

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

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NUMBER

L0461/7099

09/674593

U.S. APPLICATION NO (if known, see 37 CFR 1.5)

INTERNATIONAL APPLICATION NO.

PCT/US99/10424

INTERNATIONAL FILING DATE

13 May 1999 (13.05.99)

PRIORITY DATE CLAIMED

13 May 1998 (13.05.98)

TITLE OF INVENTION

TUMOR ASSOCIATED ANTIGEN ENCODED BY THE REVERSE STRAND OF A NEW UBIQUITOUSLY EXPRESSED GENE

APPLICANT(S) FOR DO/EO/US

VAN DEN EYNDE, Benoit; BOON-FALLEUR, Thierry

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ has been transmitted by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)) with verification of translation.
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(C)(5)).

Items 11. To 16. Below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98 with references.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification (submitted as a first Preliminary Amendment).
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
Copy of PCT Published Application page 1

Express Mail Label No. EL310414331US Mailed November 2, 2000

17. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS <small>PTO USE ONLY</small>	
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):					
Search Report has been prepared by the EPO or JPO \$860.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482) \$690.00					
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).. \$710.00					
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... \$1000.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$100.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$860.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 X 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	32- 20 =	12	X \$18.00	\$216.00	
Independent Claims	7 - 3 =	4	X \$80.00	\$320.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$	
TOTAL OF ABOVE CALCULATIONS =				\$1396.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$.00	
SUBTOTAL =				\$	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate coversheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	
TOTAL FEES ENCLOSED =				\$1396.00	
				Amount to be: refunded	\$
				charged	\$

- a. ☒ A check in the amount of \$ 1396.00 to cover the above fees is enclosed.
- b. ☐ Please charge by Deposit Account No. 23/2825 In the amount of \$ _____ To cover the above fees.
A duplicate copy of this sheet is enclosed.
- c. ☒ The commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23/2825. A duplicate of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

John R. Van Amsterdam
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210

SIGNATURE

John R. Van Amsterdam
NAME

40,212
REGISTRATION NO

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Van den Eynde and Boon-Falleur
U.S. Serial No. : 09/674,593
International Application No. : PCT/US99/10424
International Filing Date : 13 May 1999 (13.05.99)
Earliest Priority Date : 13 May 1998 (13.05.98)
Title : TUMOR ASSOCIATED ANTIGEN ENCODED BY THE
REVERSE STRAND OF A NEW UBIQUITOUSLY
EXPRESSED GENE

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to Box PCT, Commissioner for Patents, Washington, D.C. 20231, on the 17th day of May, 2001.



Monica E. Zombori

Box PCT
Commissioner for Patents
Washington, DC 20231

Sir:

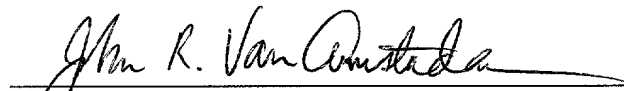
Transmitted herewith are the following documents:

- ☒ Second Preliminary Amendment
- ☒ Return Receipt Postcard

If the enclosed papers are considered incomplete, the Mail Room and/or the Application Branch is respectfully requested to contact the undersigned at (617) 720-3500, Boston, Massachusetts.

No check is enclosed. If a fee is determined to be required, the balance may be charged to the account of the undersigned, Deposit Account No. 23/2825. A duplicate of this sheet is enclosed.

Respectfully submitted,



John R. Van Amsterdam, Reg. No. 40,212
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210
Tel: (617) 720-3500

Attorney's Docket No. L0461/7099

Dated: May 17, 2001

xNDD

533796.1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Van den Eynde and Boon-Falleur
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Monica E. Zombori

Box PCT
Commissioner for Patents
Washington, DC 20231

SECOND PRELIMINARY AMENDMENT

Sir:

Please amend the United States national phase application of the above-identified PCT international application as follows.

In the Specification

Please add the following paragraph as the first paragraph of the specification following the title.

Related Applications

This application claims the benefit under 35 U.S.C. §120 or 35 U.S.C. §365(c) of PCT International application PCT/US99/10424, designating the United States of America, and filed May 13, 1999. PCT application PCT/US99/10424, of which this application is a national stage filing under 35 U.S.C. §371, was published under PCT Article 21(2) in English.

Application number PCT/US99/10424 claims the benefit under 35 U.S.C. §119(e) of United States provisional application 60/085,318, filed May 13, 1998.

Remarks

Applicants have amended the specification to provide priority application information and information regarding the publication in English under PCT Article 21(2) of the PCT application of which the above-identified application is a U.S. national stage application. No new matter has been added.

Respectfully submitted,



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Attorney's Docket No. L0461/7099

Dated: May 17, 2001

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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REVERSE STRAND OF A NEW UBIQUITOUSLY
EXPRESSED GENE

Commissioner for Patents
Box PCT
Washington, DC 20231

PRELIMINARY AMENDMENT

Dear Sir:

Please amend the application as follows, prior to calculation of the fees.

In the Claims

Please cancel claims 3-7, 10, 11, 14, 16, 20, 23, 24, 26-28, 31-34, 37, 39, 40, 42, 43, 45, 46, 49, 51-53, 55, 63 and 64 without prejudice.

Please amend the claims as follows:

- 1.(amended) An isolated nucleic acid molecule selected from the group consisting of
- (a) a nucleic acid molecule which codes for a RUR-1 sense-encoded or a RUR-1 antisense-encoded polypeptide and which hybridizes under stringent conditions to a molecule having a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO:1 and the nucleotide sequence of SEQ ID NO:4,
 - [(b) deletions, additions and substitutions of the nucleic acid molecules of (a), which code for a RUR-1 sense-encoded or a RUR-1 antisense-encoded polypeptide,]
 - [(c)] (b) nucleic acid molecules that differ from the nucleic acid molecules of (a) [or (b)] in codon sequence due to the degeneracy of the genetic code, and
 - [(d)] (c) complements of (a) and (b) [, (b) and (c)].

- 2.(amended) The isolated nucleic acid molecule of claim 1, wherein the isolated nucleic acid molecule comprises [the] a nucleotide sequence selected from the group consisting of SEQ ID NO:1, the coding region of SEQ ID NO:1, SEQ ID NO:4 and the coding region of SEQ ID NO:4.

8.(amended) An isolated nucleic acid molecule selected from the group consisting of:

(a) a unique fragment of nucleotides 1-1382 of SEQ ID NO:1 between 12 and 1381 contiguous nucleotides in length,

(b) a unique fragment of nucleotides 1-2167 of SEQ ID NO:4 between 12 and 2166 contiguous nucleotides in length,

(c) complements of ["(a)"], and

(d) complements of ["(b)"],

wherein the unique fragment excludes nucleic acid molecules which consist only of a nucleotide sequence selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:11.

9.(amended) The isolated nucleic acid molecule of claim 8, wherein the isolated nucleic acid molecule is selected from the group consisting of nucleic acid molecules [having] comprising fragments of SEQ ID NO:1 or of SEQ ID NO:4 of at least 14 contiguous nucleotides, at least 15 contiguous nucleotides, at least 16 contiguous nucleotides, at least 18 contiguous nucleotides, at least 20 contiguous nucleotides, at least 22 contiguous nucleotides and at least 25 contiguous nucleotides; nucleic acid molecules between 12 and 32 contiguous nucleotides, and nucleic acid molecules comprising at least 5 contiguous nucleotides not present in SEQ ID NO:10 or SEQ ID NO:11.

12.(amended) An expression vector comprising the isolated nucleic acid molecule of [any of] claim[s] 1[-11] operably linked to a promoter.

13.(amended) A host cell transformed or transfected with the expression vector of claim 12, optionally expressing an HLA molecule.

15.(amended) An isolated polypeptide encoded by the isolated nucleic acid molecule of [any of] claim[s] 1[-7], or a functional variant thereof having additions, deletions or substitutions in the amino acid sequence of the isolated polypeptide.

17.(amended) An isolated polypeptide comprising an amino acid sequence selected from the group consisting of the amino acid sequence of SEQ ID NO:2, the amino acid sequence of SEQ ID NO:3 and the amino acid sequence of SEQ ID NO:5.

21.(amended) An isolated polypeptide which selectively binds a protein encoded by the isolated nucleic acid molecule of [any of] claim[s] 1[-7].

22.(amended) The isolated polypeptide of claim 21, wherein the isolated polypeptide is an Fab or F(ab)₂ fragment of an antibody, a fragment of an antibody including a CDR3 region selective for the protein, or a monoclonal antibody.

30.(amended) The method of claim 29 wherein the agent is selected from the group consisting of a nucleic acid molecule comprising SEQ ID NO:1 or a unique fragment thereof, a cytolytic T lymphocyte, an antibody and an antibody fragment; or wherein the interaction is determined by amplifying at least a portion of the nucleic acid molecule, or wherein the biological sample is isolated from a tissue selected from the group consisting of non-liver tissue, non-kidney tissue, non-bladder tissue, and non-testis tissue.

35.(amended) A method for treating a subject with a disorder characterized by expression of a RUR-1 antisense cDNA-encoded tumor associated polypeptide, comprising
administering to the subject an amount of an agent which enriches selectively in the subject the presence of complexes of a HLA molecule and a tumor rejection antigen derived from [a] the RUR-1 antisense cDNA-encoded tumor associated polypeptide of claim 15,
sufficient to ameliorate the disorder.

36.(amended) The method of claim 35, wherein the agent is an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:3, or an immunogenic fragment thereof; or wherein the disorder is cancer excluding cancers selected from the group consisting of liver cancer, kidney cancer, bladder cancer, and testicular cancer.

38.(amended) A method for treating a subject with a disorder characterized by expression of a RUR-1 antisense cDNA nucleic acid molecule or an expression product thereof, comprising:

administering to the subject an amount of autologous cytotoxic T cells sufficient to ameliorate the disorder, wherein the cytotoxic T cells are specific for complexes of an HLA molecule and [a] the RUR-1 antisense cDNA-encoded tumor associated polypeptide of claim 15 or an immunogenic fragment thereof.

41.(amended) A method for treating a subject with a disorder characterized by expression of a RUR-1 antisense cDNA nucleic acid molecule or an expression product thereof, comprising:

administering to the subject an amount of [a] the RUR-1 antisense cDNA-encoded tumor associated polypeptide of claim 15 or an immunogenic fragment thereof sufficient to ameliorate the disorder.

44.(amended) A method for enriching selectively a population of T cells with cytotoxic T cells specific for a RUR-1 antisense cDNA-encoded tumor associated polypeptide comprising:

contacting an isolated population of T cells with an agent presenting a complex of [a] the RUR-1 antisense cDNA-encoded tumor associated polypeptide of claim 15 or an immunogenic fragment thereof and a HLA presenting molecule in an amount sufficient to selectively enrich the isolated population of T cells with the cytotoxic T cells.

47.(amended) A vaccine composition comprising

(a) a nucleic acid encoding a RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof;

(b) a RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof; or

(c) a cell which expresses a RUR-1 antisense cDNA nucleic acid or polypeptide, or an immunogenic fragment thereof.

48.(amended) The vaccine composition of claim 47, wherein:

(a) the composition further comprises a nucleic acid encoding a second tumor associated polypeptide or an immunogenic fragment thereof which is a non-RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof;

(b) the composition further comprises a second tumor associated polypeptide or an immunogenic fragment thereof which is a non-RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof; or

(c) the cell further comprises a second tumor associated nucleic acid or polypeptide, which is a non-RUR-1 antisense cDNA nucleic acid or tumor associated polypeptide, or an immunogenic fragment thereof.

50.(amended) The vaccine composition of claim 47 [49], wherein the RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof comprises the amino acid sequence of SEQ ID NO:3.

54.(amended) The vaccine composition of [any of] claim[s] 47[-53], further comprising an adjuvant or a pharmaceutically acceptable carrier.

61.(amended) The method of claim 60 wherein the agent is selected from the group consisting of a nucleic acid molecule comprising SEQ ID NO:1 or a unique fragment thereof; a cytolytic T lymphocyte; and an antibody or antibody fragment.

Remarks

Applicants have amended certain claims to eliminate multiple dependencies and/or clarify claim language. Subject matter added to the claims is supported in the claims as filed and in the specification. No new matter has been added.

Respectfully submitted,



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Attorney's Docket No. L0461/7099

Dated: November 2, 2000

X11/13/00

491806.1

**TUMOR ASSOCIATED ANTIGEN ENCODED BY THE REVERSE STRAND OF A
NEW UBIQUITOUSLY EXPRESSED GENE**

Field of the Invention

5 This invention relates to nucleic acid molecules and encoded polypeptides which are expressed preferentially in tumors. The nucleic acid molecules and encoded polypeptides are useful in, *inter alia*, diagnostic and therapeutic contexts.

Background of the Invention

10 The phenotypic changes which distinguish a tumor cell from its normal counterpart are often the result of one or more changes to the genome of the cell. The genes which are expressed in tumor cells, but not in normal counterparts, can be termed "tumor associated" genes. These tumor associated genes are markers for the tumor phenotype. The expression of tumor associated genes can also be an essential event in the process of tumorigenesis.

15 Typically, the host recognizes as foreign the tumor associated genes which are not expressed in normal non-tumorigenic cells. Thus, the expression of tumor associated genes can provoke an immune response against the tumor cells by the host. Tumor associated genes can also be expressed in normal cells within certain tissues without provoking an immune response. In such tissues, expression of the gene and/or presentation of an ordinarily
20 immunologically recognizable fragment of the protein product on the cell surface may not provoke an immune response because the immune system does not "see" the cells inside these immunologically privileged tissues. Examples of immunologically privileged tissues include brain, retina and testis.

The discovery of tumor associated expression of a nucleic acid or polypeptide provides
25 a means of identifying a cell as a tumor cell. Diagnostic compounds can be based on the tumor associated nucleic acid or polypeptide, and used to determine the presence and location of tumor cells. Further, when the tumor associated nucleic acid or polypeptide is essential for an aspect of the tumor phenotype (e.g., unregulated growth or metastasis), the tumor associated nucleic acid or polypeptide can be used to provide therapeutics such as antisense
30 nucleic acids which can reduce or substantially eliminate expression of that nucleic acid or polypeptide, thereby reducing or substantially eliminating the phenotypic aspect which depends on the expression of the particular tumor associated nucleic acid or polypeptide.

As previously noted, the polypeptide products of tumor associated genes can be the targets for host immune surveillance and provoke selection and expansion of one or more clones of cytotoxic T lymphocytes specific for the tumor associated gene product. Examples of this phenomenon include proteins and fragments thereof encoded by the MAGE family of genes, the tyrosinase gene, the Melan-A gene, the BAGE gene, the GAGE gene, the RAGE family of genes, the PRAME gene and the brain glycogen phosphorylase gene, as are detailed below. Thus, tumor associated expression of nucleic acids or polypeptides suggests that such nucleic acids or polypeptides can encode proteins or peptides which will be recognized by the immune system as foreign and thus provide a target for tumor rejection. Such nucleic acids encode "tumor rejection antigen precursors", or TRAPs, which may be used to generate therapeutics for enhancement of the immune system response to tumors expressing such genes and proteins.

The process by which the mammalian immune system recognizes and reacts to foreign or alien materials is a complex one. An important facet of the system is the T cell response. This response requires that T cells recognize and interact with complexes of cell surface molecules, referred to as human leukocyte antigens ("HLA"), or major histocompatibility complexes ("MHCs"), and peptides. The peptides are derived from larger molecules which are processed by the cells which also present the HLA/MHC molecule. See in this regard Male et al., Advanced Immunology (J.P. Lipincott Company, 1987), especially chapters 6-10. The interaction of T cells and complexes of HLA/peptide is restricted, requiring a T cell specific for a particular combination of an HLA molecule and a peptide. If a specific T cell is not present, there is no T cell response even if its partner complex is present. Similarly, there is no response if the specific complex is absent, but the T cell is present. The mechanism is involved in the immune system's response to foreign materials, in autoimmune pathologies, and in responses to cellular abnormalities. Much work has focused on the mechanisms by which proteins are processed into the HLA binding peptides. See, in this regard, Barinaga, *Science* 257: 880, 1992; Fremont et al., *Science* 257: 919, 1992; Matsumura et al., *Science* 257: 927, 1992; Latron et al., *Science* 257: 964, 1992.

The mechanism by which T cells recognize cellular abnormalities has also been implicated in cancer. For example, in PCT application PCT/US92/04354, filed May 22, 1992, published on November 26, 1992, and incorporated by reference, a family of genes is disclosed, which are processed into peptides which, in turn, are expressed on cell surfaces,

which can lead to lysis of the tumor cells by specific CTLs. The genes are said to code for "tumor rejection antigen precursors" or "TRAP" molecules, and the peptides derived therefrom are referred to as "tumor rejection antigens" or "TRAs". See Traversari et al., *J. Exp. Med.* 176:1453-1457, 1992; van der Bruggen et al., *Science* 254: 1643, 1991; De Plaen et al., *Immunogenetics* 40:360-369, 1994 for further information on this family of genes. Also, see U.S. Patent No. 5,342,774.

In U.S. Patent No. 5,405,940, the disclosure of which is incorporated by reference, nonapeptides are taught which are presented by the HLA-A1 molecule. The reference teaches that given the known specificity of particular peptides for particular HLA molecules, one should expect a particular peptide to bind one HLA molecule, but not others. This is important, because different individuals possess different HLA phenotypes. As a result, while identification of a particular peptide as being a partner for a specific HLA molecule has diagnostic and therapeutic ramifications, these are only relevant for individuals with that particular HLA phenotype. There is a need for further work in the area, because cellular abnormalities are not restricted to one particular HLA phenotype, and targeted therapy requires some knowledge of the phenotype of the abnormal cells at issue.

In U.S. Patent 5,629,166, incorporated by reference, the fact that the MAGE-1 expression product is processed to a second TRA is disclosed. This second TRA is presented by HLA-Cw16 molecules, also known as HLA-Cw*1601. The disclosure shows that a given TRAP can yield a plurality of TRAs.

In U.S. Patent 5,487,974 incorporated by reference herein, tyrosinase is described as a tumor rejection antigen precursor. This reference discloses that a molecule which is produced by some normal cells (e.g., melanocytes), is processed in tumor cells to yield a tumor rejection antigen that is presented by HLA-A2 molecules.

In U.S. patent 5,620,886, incorporated herein by reference in its entirety, a second TRA, not derived from tyrosinase is taught to be presented by HLA-A2 molecules. The TRA is derived from a TRAP, but is coded for by a known MAGE gene. This disclosure shows that a particular HLA molecule may present TRAs derived from different sources.

In U.S. Patent 5,571,711 and U.S. Patent 5,683,886, the entire disclosures of which are incorporated herein by reference, an unrelated tumor rejection antigen precursor, the so-called "BAGE" precursor, is described. TRAs are derived from the TRAP and also are described. They form complexes with MHC molecule HLA-C-Clone 10, renamed later as HLA-

Cw*1601.

In U.S. Patent 5,648,226 and U.S. Patent 5,610,013, the entire disclosures of which are incorporated herein by reference, another unrelated tumor rejection antigen precursor, the so-called "GAGE" precursor, is described. The GAGE precursor is not related to the BAGE or the MAGE family.

In PCT publication WO96/29409, published September 26, 1996, and entitled "RAGE Tumor Rejection Antigens", incorporated herein by reference in its entirety, a family of TRAPs is taught which are not derived from any of the foregoing genes. The TRAPs are referred to as the RAGE family of genes. In addition, a TRA derived from one member of the RAGE family of genes is taught to be presented by HLA-B7 molecules. This disclosure shows that additional TRAPs and TRAs can be derived from different sources.

In U.S. Patent 5,589,334, incorporated herein by reference in its entirety, another TRAP is taught which is not derived from any of the foregoing genes. The gene encoding the TRAP is referred to as MUM-1. A tumor rejection antigen, LB-33, is described in the application.

In U.S. patent application no. 08/373,636, filed January 17, 1995, and entitled "Isolated Nucleic Acid Molecule Which Codes for a Tumor Rejection Antigen Precursor Which is Processed to Antigens Presented by HLA Molecules and Uses Thereof", incorporated herein by reference in its entirety, other TRAPs are taught which are derived from LB33 and presented by HLA-B13, HLA-Cw6, HLA-A28 and HLA-A24.

In PCT publication WO96/10577, published April 11, 1996, and entitled "Isolated Nucleic Acid Molecule Coding for a Tumor Rejection Antigen Precursor DAGE and Uses Thereof", incorporated herein by reference in its entirety, another TRAP is taught which is not derived from any of the foregoing genes. The TRAP was referred to as DAGE, but is now referred to as PRAME. A tumor rejection antigen is described in the application which is presented by HLA-A24.

In U.S. patent 5,821,122, incorporated herein by reference in its entirety, another TRAP is taught which is not derived from any of the foregoing genes. The TRAP is referred to as NAG. Various TRAs derived from NAG and presented by HLA-A2 are taught in this application.

In U.S. Patent 5,587,289, incorporated herein by reference in its entirety, three TRAPs are taught which are not derived from any of the foregoing genes. These TRAPs were

- 5 -

referred to as MAGE-Xp2, MAGE-Xp3 and MAGE-Xp4, but are now referred to as MAGE-B genes.

The work which is presented by the papers, patents and patent applications described above deal, for the most part, with the MAGE family of genes, the BAGE gene, the GAGE gene and the RAGE family of genes.

Since the individual tumor antigens presently known may be expressed only in a fraction of tumors, the availability of additional tumor antigens would significantly enlarge the proportion of patients who are potentially eligible for therapeutic interventions. Thus there presently is a need for additional tumor antigens for development of therapeutics and diagnostics applicable to a greater number of cancer patients having various cancers.

The invention is elaborated upon further in the disclosure which follows.

Summary of the Invention

It now has been discovered that the reverse strand of an ubiquitously expressed gene, RUR-1, which is unrelated to any of the foregoing TRAPs, is expressed in a tumor associated pattern. The invention provides isolated nucleic acid molecules derived from the forward and reverse strands of the gene, some of which encode tumor associated polypeptides. The invention also provides expression vectors containing those molecules and host cells transfected with those molecules, as well as isolated polypeptides encoded by the nucleic acid molecules (including tumor rejection antigen precursors and fragments of the isolated polypeptides). The foregoing isolated nucleic acid molecules and polypeptides can be used in the diagnosis, prognosis or treatment of conditions characterized by the expression of a tumor associated gene.

According to one aspect of the invention, an isolated nucleic acid molecule is provided. The isolated nucleic acid molecule is selected from the group consisting of a nucleic acid molecule which hybridizes under stringent conditions to a molecule having a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO:1 and the nucleotide sequence of SEQ ID NO:4, wherein the isolated nucleic acid molecule codes for a RUR-1 sense-encoded or a RUR-1 antisense-encoded polypeptide, nucleic acid molecules that differ from the nucleic acid molecules of the foregoing nucleic acid molecules in codon sequence due to the degeneracy of the genetic code, and complements of any of the foregoing nucleic acid molecules. In certain embodiments, the

isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:1, the coding region of the nucleotide sequence of SEQ ID NO:1, the nucleic acid sequence of SEQ ID NO:4, or the coding region of the nucleic acid sequence of SEQ ID NO:4. In preferred embodiments, the isolated nucleic acid molecule consists of the nucleotide sequence of SEQ ID NO:1 or the nucleotide sequence of SEQ ID NO:4.

According to another aspect of the invention, an isolated nucleic acid molecule is provided which is a unique fragment of nucleotides 1-1382 of SEQ ID NO:1 between 12 and 1381 contiguous nucleotides in length, a unique fragment of nucleotides 1-2167 of SEQ ID NO:4 between 12 and 2166 contiguous nucleotides in length, or a complement of the foregoing unique fragments. The unique fragment excludes nucleic acid molecules which consist only of a nucleotide sequence set forth in SEQ ID NO:10 or SEQ ID NO:11. In some embodiments the unique fragment is a nucleic acid molecule having at least 14 contiguous nucleotides, 15 contiguous nucleotides, 16 contiguous nucleotides, 18 contiguous nucleotides, 20 contiguous nucleotides, 22 contiguous nucleotides, 25 contiguous nucleotides or more of the aforementioned nucleic acid molecules. Each and every fragment of the nucleic acid molecules of 12 or greater nucleotides up to the entire length of the nucleic acid molecules is embraced by the invention. In other embodiments, the unique fragment is a nucleic acid molecule having between 12 and 32 contiguous nucleotides of the foregoing nucleic acid molecules. Preferably the isolated nucleic acid molecule includes at least 5 contiguous nucleotides not present in SEQ ID NO:10 or SEQ ID NO:11.

According to still other aspects of the invention, expression vectors are provided which include the isolated nucleic acid molecule of any of the foregoing nucleic acids, operably linked to a promoter. Host cells transformed or transfected with the expression vectors also are provided. Optionally the host cell expresses an HLA molecule.

According to yet another aspect of the invention, an isolated polypeptide is provided. The isolate polypeptide is encoded by any of the foregoing isolated nucleic acid molecules. Functional variants of the isolated polypeptides also are provided. In certain embodiments, the isolated polypeptide has an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:5.

In another aspect of the invention, an isolated polypeptide is provided which includes the amino acid sequence of SEQ ID NO:3.

According to another aspect of the invention, an isolated polypeptide is provided

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which is a unique fragment of SEQ ID NO:2 between 9 and 83 amino acids in length, or a unique fragment of SEQ ID NO:5 between 9 and 475 amino acids in length. In certain embodiments, the unique fragment binds to a polypeptide-binding agent, preferably an antibody or a cytotoxic T lymphocyte.

5 According to still another aspect of the invention an isolated polypeptide is provided which selectively binds a polypeptide encoded by the foregoing isolated nucleic acid molecules. In certain embodiments, the isolated polypeptide is an Fab or F(ab)₂ fragment of an antibody, a fragment of an antibody which includes a CDR3 region selective for the polypeptide, or a monoclonal antibody.

10 The invention in another aspect provides a kit for detecting the presence of the expression of a nucleic acid which encodes a tumor associated polypeptide precursor. The kit includes a pair of isolated nucleic acid molecules each of which consists of a molecule selected from the group consisting of a 12-32 nucleotide contiguous segment of nucleotides 1-1382 of SEQ ID NO:1, and complements thereof, wherein the contiguous segments are
15 nonoverlapping. In some embodiments the pair of isolated nucleic acid molecules is constructed and arranged to selectively amplify an isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1. In certain preferred embodiments, the pair of isolated nucleic acid molecules is SEQ ID NO:8 and SEQ ID NO:9. In other preferred embodiments, the pair of isolated nucleic acid molecules is PCR primers, wherein one of the
20 primers is a unique fragment of SEQ ID NO:1.

According to still another aspect of the invention, methods for diagnosing a disorder characterized by expression of a RUR-1 antisense cDNA nucleic acid molecule or an expression product thereof are provided. The methods include contacting a biological sample isolated from a subject with an agent that selectively binds the isolated RUR-1 antisense
25 cDNA nucleic acid molecule of claim 1 or an expression product thereof, and determining the interaction between the agent and the nucleic acid molecule or the expression product as a determination of the disorder. In certain embodiments, the agent is a nucleic acid molecule comprising SEQ ID NO:1 or a unique fragment thereof, a cytolytic T lymphocyte, or an antibody or antibody fragment. In other embodiments, the interaction is determined by
30 amplifying at least a portion of the nucleic acid molecule. In still other embodiments, the biological sample is isolated from a tissue selected from the group consisting of non-liver tissue, non-kidney tissue, non-bladder tissue, and non-testis tissue.

According to yet another aspect of the invention, methods for treating a subject with a disorder characterized by expression of a RUR-1 antisense cDNA-encoded tumor associated polypeptide are provided. The methods include administering to the subject an amount of an agent which enriches selectively in the subject the presence of complexes of a HLA molecule and a tumor rejection antigen derived from a RUR-1 antisense cDNA-encoded tumor associated polypeptide, sufficient to ameliorate the disorder. In some embodiments, the agent is an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:3, an immunogenic fragment thereof, or a functional variant thereof. In other embodiments, the disorder is cancer, excluding liver cancer, kidney cancer, bladder cancer, and testicular cancer.

According to another aspect of the invention, methods for treating a subject having a disorder characterized by expression of a RUR-1 antisense cDNA nucleic acid molecule or an expression product thereof are provided. The methods include administering to the subject an amount of autologous cytotoxic T cells sufficient to ameliorate the disorder. The cytotoxic T cells are specific for complexes of an HLA molecule and a RUR-1 antisense cDNA-encoded tumor associated polypeptide, an immunogenic fragment thereof, or a functional variant thereof. In some embodiments, the RUR-1 antisense cDNA-encoded tumor associated polypeptide comprises the amino acid sequence of SEQ ID NO:3. In certain embodiments the disorder is cancer, preferably excluding liver cancer, kidney cancer, bladder cancer, and testicular cancer.

The invention also provides, in another aspect, methods for treating a subject with a disorder characterized by expression of a RUR-1 antisense cDNA nucleic acid molecule or an expression product thereof. The methods include administering to the subject an amount of a RUR-1 antisense cDNA-encoded tumor associated polypeptide, or an immunogenic fragment thereof, or a functional variant thereof, sufficient to ameliorate the disorder. The RUR-1 antisense cDNA-encoded tumor associated polypeptide includes the amino acid sequence of SEQ ID NO:3 in certain embodiments. In other embodiments, the disorder is cancer, preferably excluding liver cancer, kidney cancer, bladder cancer, and testicular cancer.

Also provided are methods for enriching selectively a population of T cells with cytotoxic T cells specific for a RUR-1 antisense cDNA-encoded tumor associated polypeptide. The methods include contacting an isolated population of T cells with an agent presenting a complex of a RUR-1 antisense cDNA-encoded tumor associated polypeptide, an immunogenic fragment thereof or a functional variant thereof, and a HLA presenting

molecule in an amount sufficient to selectively enrich the isolated population of T cells with the cytotoxic T cells. In certain embodiments the RUR-1 antisense cDNA-encoded tumor associated polypeptide includes the amino acid sequence of SEQ ID NO:3. Preferably the agent is a cell which expresses a RUR-1 antisense cDNA-encoded tumor associated polypeptide and a HLA molecule.

In all of the foregoing methods, the amount administered is an effective amount.

According to other aspects of the invention, vaccine compositions are provided. The vaccine compositions include nucleic acids encoding a RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof, polypeptides or fragments encoded by the foregoing nucleic acids, or cells which expresses the foregoing nucleic acids, polypeptides, or immunogenic fragments thereof. Preferably the RUR-1 antisense cDNA-encoded tumor associated polypeptide or immunogenic fragment thereof includes the amino acid sequence of SEQ ID NO:3. The vaccine compositions in certain embodiments further include a nucleic acid encoding a second tumor associated polypeptide or an immunogenic fragment thereof which is a non-RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof. In other embodiments, the vaccine compositions include a second tumor associated polypeptide or an immunogenic fragment thereof which is a non-RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof. In still other embodiments, any of the foregoing vaccine compositions also include an adjuvant and/or a pharmaceutically acceptable carrier.

According to another aspect of the invention, an isolated nucleic acid molecule is provided which includes a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:3, or an immunogenic fragment thereof. In some embodiments, the nucleic acid molecule also includes a nucleotide sequence encoding a second tumor associated polypeptide or an immunogenic fragment thereof which is a non-RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof.

According to yet other aspects of the invention, compositions are provided which include an isolated RUR-1 sense or RUR-1 antisense nucleic acid, or a polypeptide encoded by those nucleic acids and a pharmaceutically acceptable carrier.

The invention provides in another aspect methods for determining the prognosis of a disorder characterized by expression of a RUR-1 antisense nucleic acid molecule or an

expression product thereof. The methods include the steps of (a) contacting a biological sample isolated from a subject at a first time with an agent that selectively binds the isolated RUR-1 antisense nucleic acid molecule of claim 1 or an expression product thereof, (b) determining the interaction between the agent and the nucleic acid molecule or the expression product as a determination of the state of the disorder at the first time, (c) contacting a second biological sample isolated from the subject at a second time with the agent, (d) determining the interaction between the agent and the nucleic acid molecule or the expression product in the second biological sample as a determination of the state of the disorder at the second time, and (e) comparing the state of the disorder at the first time and the second time as a determination of the prognosis. In certain embodiments, the agent is a nucleic acid molecule including the nucleotide sequence of SEQ ID NO:1, or a unique fragment thereof, or a cytolytic T lymphocyte, or an antibody or antibody fragment. In other embodiments, the interactions are determined by amplifying at least a portion of the nucleic acid molecule. In still other embodiments, the biological samples are isolated from a tissue selected from the group consisting of non-liver tissue, non-kidney tissue, non-bladder tissue, and non-testis tissue.

Use of the foregoing compositions in the preparation of medicaments is also provided. In particular, use of the composition in the preparation of a medicament for treating cancer is provided.

Functional variants of the foregoing nucleic acid molecules, polypeptides, and immunogenic fragments, including variants comprising additions, substitutions and deletions of nucleotide or amino acid sequences, also are embraced by the invention.

These and other objects of the invention will be described in further detail in connection with the detailed description of the invention.

Brief Description of the Figures

Figure 1 shows specific lysis of autologous tumor cells LE9211-RCC by CTL clone 361A/21.

Figure 2 depicts the isolation of a cDNA clone encoding the HLA-B7-restricted antigen recognized by CTL clone 361A/21.

Figure 3 shows lysis by CTL 361A/21 of HLA-B7-positive LB23-SAR sarcoma cells after stable transfection with an expression plasmid containing cDNA 4.1 (RUR-1 antisense

cDNA).

Figure 4 shows lysis by CTL 361A/21 of autologous EBV-transformed B cells incubated with peptide LPRWPPPQL (SEQ ID NO:3).

Figure 5 depicts the sequence of RUR-1 antisense cDNA and of the protein predicted by the open reading frame. The antigenic peptide recognized by CTL 361A/21 is underlined. The vertical arrows indicate the limits of the fragment that is identical to the reverse strand of the first exon of the RUR-1 gene. The horizontal arrows indicate the primers used for testing the expression of RUR-1 antisense cDNA by RT-PCR.

Figure 6 shows the sequence of cDNA I.1 (RUR-1 sense cDNA) and of the protein predicted by the open reading frame. The vertical arrow indicates the limit between exons 1 and 2. The horizontal arrows indicate the primers used for testing the expression of RUR-1 sense cDNA by RT-PCR.

Figure 7 depicts the partial structure of the RUR-1 gene showing the sense (I.1) and the antisense (4.1) transcripts.

Figure 8. Correlation between expression of RUR-1 antisense transcript and recognition by CTL 361A/21. (A) Lysis by CTL 361A/21 of two HLA-B7-positive renal cell carcinoma (RCC) lines expressing the RUR-1 antisense transcript (4.1). (B) Stimulation of CTL 361A/21 TNF release by melanoma cell lines derived from 4 different HLA-B7 patients.

Brief Description of the Sequences

SEQ ID NO:1 is the nucleotide sequence of the RUR-1 antisense (4.1) cDNA clone.

SEQ ID NO:2 is the amino acid of the polypeptide encoded by the RUR-1 antisense cDNA.

SEQ ID NO:3 is the amino acid of the tumor rejection antigen derived from the RUR-1 antisense-encoded polypeptide.

SEQ ID NO:4 is the nucleotide sequence of the RUR-1 sense (I.1) cDNA clone.

SEQ ID NO:5 is the amino acid of the polypeptide encoded by the RUR-1 cDNA.

SEQ ID NO:6 is the nucleotide sequence of the VDE87 primer.

SEQ ID NO:7 is the nucleotide sequence of the VDE93 primer.

SEQ ID NO:8 is the nucleotide sequence of the VDE119 primer.

SEQ ID NO:9 is the nucleotide sequence of the VDE120 primer.

SEQ ID NO:10 is the nucleotide sequence of the EST AA863443.

SEQ ID NO:11 is the nucleotide sequence of the EST AA004587.

Detailed Description of the Invention

The examples which follow show the isolation of nucleic acid molecules which code
5 for polypeptides and are expressed preferentially in tumor samples and tumor-derived cell
lines. These isolated nucleic acid molecules, however, are not homologous with any of the
previously disclosed coding sequences described in the references set forth *supra*. Hence, one
aspect of the invention is an isolated nucleic acid molecule which includes all or a unique
portion of the nucleotide sequence set forth in SEQ ID NO:1, referred to herein as "cDNA
10 4.1", "RUR-1 antisense" or "RUR-1 antiparallel" cDNA or nucleic acids, "RUR-1 reverse
strand" and the like. This sequence is not any of the MAGE, BAGE, GAGE, RAGE,
LB33/MUM-1, PRAME, NAG, or MAGE-B sequences, as will be seen by comparing them to
the sequence of any of the genes described in the references. It was determined that the
nucleic acid set forth in SEQ ID NO:1 represents, quite unexpectedly, the antiparallel or
15 reverse strand of a ubiquitously expressed gene. The nucleotide of this ubiquitously
expressed gene, referred to herein as "cDNA I.1", "RUR-1 sense cDNA", "RUR-1" and the
like, is provided as SEQ ID NO:4. Therefore, also provided herein are nucleic acids which
include all or a portion of the nucleotide sequence set forth in SEQ ID NO:4.

The invention thus involves RUR-1 sense and antisense nucleic acids, polypeptides
20 encoded by those nucleic acids, functional homologs, modifications and variants of the
foregoing, useful fragments of the foregoing, as well as therapeutics and diagnostics related
thereto.

Also a part of the invention are those nucleic acid sequences which also code for a
RUR-1-encoded polypeptide or a RUR-1 antisense-encoded polypeptide and which hybridize
25 to a nucleic acid molecule consisting of the nucleotide sequence set forth in SEQ ID NO:1
(RUR-1 antisense cDNA) or SEQ ID NO:4 (RUR-1 sense cDNA), under stringent conditions.
Complements of the foregoing are also embraced by the invention.

Such nucleic acids, which are tumor associated polypeptide precursors in the case of
RUR-1 antisense cDNA, and may be DNA, RNA, or composed of mixed
30 deoxyribonucleotides and ribonucleotides. The nucleic acids can also incorporate synthetic
non-natural nucleotides. A tumor associated nucleic acid or polypeptide is a nucleic acid or
polypeptide expressed preferentially in tumor cells. Various methods for determining the

expression of a nucleic acid and/or a polypeptide in normal and tumor cells are known to those of skill in the art and are described further below. As used herein, tumor associated polypeptides include proteins, protein fragments, and peptides. In particular, tumor associated polypeptides include TRAPs and TRAs.

5 The term "stringent conditions" as used herein refers to parameters with which the art is familiar. More specifically, stringent conditions, as used herein, refers to hybridization at 65°C in hybridization buffer (3.5 x SSC, 0.02% Ficoll, 0.02% polyvinyl pyrrolidone, 0.02% Bovine Serum Albumin, 25mM NaH₂PO₄ (pH 7), 0.5% SDS, 2mM EDTA). SSC is 0.15M sodium chloride/0.015M sodium citrate, pH 7; SDS is sodium dodecyl sulphate; and EDTA is
10 ethylenediaminetetracetic acid. After hybridization, the membrane upon which the nucleic acid is transferred is washed at 2 x SSC at room temperature and then at 0.1 x SSC/0.1 x SDS at 65°C.

There are other conditions, reagents, and so forth which can be used, which result in the same degree of stringency (see, e.g. *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York). The skilled artisan will be familiar with such conditions, and thus they are not given here. It will be understood, however, that the skilled
15 artisan will be able to manipulate the conditions in a manner to permit the clear identification of homologs and alleles of the RUR-1 sense and antisense nucleic acid molecules of the invention. The skilled artisan also is familiar with the methodology for screening cells, preferably cancer cells, and libraries for expression of such molecules which then are routinely isolated, followed by isolation of the pertinent nucleic acid and sequencing. Preferred homologs and alleles are those which have at least about 75% identity, preferably at
20 least about 90% identity, more preferably at least about 95% identity, and most preferably at least about 99% identity with the RUR-1 sense and antisense nucleic acid molecules.

The nucleic acids disclosed herein are useful for determining the expression of RUR-1 antisense or sense nucleic acids according to standard hybridization procedures. The nucleic acids also can be used to express RUR-1 sense-encoded or RUR-1 antisense-encoded
30 polypeptides *in vitro* or *in vivo*. The nucleic acids also can be used to prepare fragments of RUR-1 sense-encoded or RUR-1 antisense-encoded polypeptides useful for e.g., preparation of antibodies. Many other uses will be apparent to the skilled artisan.

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In screening for RUR-1 sense or antisense nucleic acid family members, a Southern blot may be performed using the foregoing conditions, together with a radioactive probe. Preferably hybridizations are performed using probes comprising the coding regions of RUR-1 antisense cDNA or RUR-1 sense cDNA, or portions thereof. After washing the
5 membrane to which the nucleic acid is finally transferred, the membrane can be placed against x-ray film to detect the radioactive signal.

The invention also includes degenerate nucleic acids which include alternative codons to those present in the native materials. For example, serine residues are encoded by the codons TCA, AGT, TCC, TCG, TCT and AGC. Each of the six codons is equivalent for the
10 purposes of encoding a serine residue. Thus, it will be apparent to one of ordinary skill in the art that any of the serine-encoding nucleotide triplets may be employed to direct the protein synthesis apparatus, *in vitro* or *in vivo*, to incorporate a serine residue. Similarly, nucleotide sequence triplets which encode other amino acid residues include, but are not limited to:
15 CCA, CCC, CCG and CCT (proline codons); CGA, CGC, CGG, CGT, AGA and AGG (arginine codons); ACA, ACC, ACG and ACT (threonine codons); AAC and AAT (asparagine codons); and ATA, ATC and ATT (isoleucine codons). Other amino acid residues may be encoded similarly by multiple nucleotide sequences. Thus, the invention embraces degenerate nucleic acids that differ from the biologically isolated nucleic acids in codon sequence due to the degeneracy of the genetic code.

20 The invention also provides isolated unique fragments of SEQ ID NO:1 or SEQ ID NO:4, or complements of SEQ ID NO:1 or SEQ ID NO:4. A unique fragment is one that is a 'signature' for the larger nucleic acid. It, for example, is long enough to assure that its precise sequence is not found in molecules outside of the RUR-1 gene family, i.e. those molecules which hybridize to RUR-1 cDNA and/or RUR-1 antisense cDNA and encode related proteins.
25 Unique fragments can be used as probes in Southern blot assays to identify family members or can be used in amplification assays such as those employing PCR. As known to those skilled in the art, large probes such as 200 nucleotides or more (e.g., 200, 250, 300, 400, 500 nucleotides) are preferred for certain uses such as Southern blots, while smaller fragments will be preferred for uses such as PCR. Unique fragments also can be used to produce fusion
30 proteins for generating antibodies or peptides, or for generating immunoassay components. Unique fragments further can be used as antisense molecules to inhibit the expression of the RUR-1 sense-encoded and/or RUR-1 antisense-encoded proteins of the invention, particularly

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for therapeutic purposes as described in greater detail below.

As will be recognized by those skilled in the art, the size of the unique fragment will depend upon its conservancy in the genetic code. Thus, some regions of SEQ ID NO:1 or SEQ ID NO:4, and their complements, will require longer segments to be unique while others will require only short segments, typically between 12 and 32 nucleotides (e.g. 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32 nucleotides long). Virtually any segment of SEQ ID NO:1 or SEQ ID NO:4, or their complements, that is 18 or more nucleotides in length will be unique. Unique fragments of RUR-1 antisense, however, exclude fragments completely composed of the nucleotide sequence of SEQ ID NO:10 (an EST having GenBank accession number AA863443) which overlaps SEQ ID NO:1. Unique fragments of RUR-1 sense exclude fragments completely composed of the nucleotide sequence of SEQ ID NO:11 (an EST having GenBank accession number AA004587) which overlaps SEQ ID NO:4. A fragment which is completely composed of the sequence of SEQ ID NO:10 or SEQ ID NO:11 is one which does not include any of the nucleotides unique to RUR-1 antisense or RUR-1 sense nucleic acids, respectively. In certain embodiments, unique fragments of RUR-1 antisense or RUR-1 sense nucleic acids include at least 5 contiguous nucleotides which are not present in SEQ ID NO:10 or SEQ ID NO:11 (e.g. which are present in SEQ ID NO:1 or SEQ ID NO:4); preferred unique fragments are those which have 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, or more nucleotides which are not present in SEQ ID NO:10 or SEQ ID NO:11. Those skilled in the art are well versed in methods for selecting such sequences, typically on the basis of the ability of the unique fragment to selectively distinguish the sequence of interest from non-family members. A comparison of the sequence of the RUR-1 antisense or RUR-1 sense nucleic acids fragment to other sequences deposited in known databases typically is all that is necessary, although *in vitro* confirmatory hybridization and sequencing analysis may be performed.

In one embodiment of the invention, isolated nucleic acid molecules include primers useful for nucleic acid amplification assays such as polymerase chain reaction (PCR). For any pair of PCR primers constructed and arranged to selectively amplify, for example, a RUR-1 antisense cDNA nucleic acid, a RUR-1 antisense cDNA specific primer may be used. Such a primer is a contiguous stretch of RUR-1 antisense cDNA which hybridizes selectively to RUR-1 antisense cDNA and not other nucleic acids. Such a specific primer would fully hybridize to a contiguous stretch of nucleotides only in RUR-1 antisense cDNA, but would

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hybridize at most only in part to genes that do not share the nucleotides to which the RUR-1 antisense cDNA specific primer binds. For efficient PCR priming and RUR-1 antisense cDNA identification, a RUR-1 antisense cDNA specific primer should be constructed and arranged so it does not hybridize efficiently at its 3' end to genes other than RUR-1 antisense cDNA. The kinetics of hybridization then will strongly favor hybridization at the 5' end. In this instance, 3' initiated PCR extension will occur only when both the 5' and 3' ends hybridize to the nucleic acid. Primers specific for RUR-1 sense cDNA similarly can be selected. Preferably the area of non-identity is at least one to four nucleotides in length and forms the 3' end of the RUR-1 antisense cDNA or RUR-1 sense cDNA specific primer. Such a primer would be perfectly complementary and contiguous with its complement in one of the RUR-1 antisense cDNA or the RUR-1 sense cDNA nucleic acids. The mismatch generated at the 3' end of the primer when hybridized to genes other than RUR-1 (sense or antisense) would preclude efficient amplification of those genes. Exemplary primers include those described in the Sequence Listing and the Examples below. Other primers can be prepared by one of skill in the art by comparison of the sequences of SEQ ID NO:1 or SEQ ID NO:4 with each other and with known sequences deposited in databases. Other exemplary primers can differ from the above by addition or deletion of 1, 2, 3, 4, 5, or more nucleotides from the 5' end of the primer. In certain cases, the primers chosen to amplify the RUR-1 antisense cDNA or RUR-1 sense cDNA nucleic acid molecules provide amplified products which are readily distinguishable by molecular size. This size difference may be distinguished readily using standard methods in the art including agarose and acrylamide gel electrophoresis. Additional methods which can distinguish nucleotide sequences of substantial homology, such as ligase chain reaction ("LCR") and other methods, will be apparent to skilled artisans.

As used herein with respect to nucleic acids, the term "isolated" means: (i) amplified *in vitro* by, for example, polymerase chain reaction (PCR); (ii) recombinantly produced by cloning; (iii) purified, as by cleavage and gel separation; or (iv) synthesized by, for example, chemical synthesis. An isolated nucleic acid is one which is readily manipulable by recombinant DNA techniques well known in the art. Thus, a nucleotide sequence contained in a vector in which 5' and 3' restriction sites are known or for which polymerase chain reaction (PCR) primer sequences have been disclosed is considered isolated but a nucleic acid sequence existing in its native state in its natural host is not. An isolated nucleic acid may be substantially purified, but need not be. For example, a nucleic acid that is isolated within a

cloning or expression vector is not pure in that it may comprise only a tiny percentage of the material in the cell in which it resides. Such a nucleic acid is isolated, however, as the term is used herein because it is readily manipulable by standard techniques known to those of ordinary skill in the art. An isolated nucleic acid molecule as used herein is not a naturally occurring chromosome.

The invention also provides isolated polypeptides, including unique fragments, encoded by RUR-1 antisense nucleic acids or RUR-1 sense nucleic acids, such as SEQ ID NO:2 or SEQ ID NO:5. Such polypeptides are useful, for example, alone or as fusion proteins to generate antibodies, as TRA peptides (e.g. SEQ ID NO:3), as a components of an immunoassay, or for determining the RUR-1 or RUR-1 antisense protein binding specificity of HLA molecules and/or CTL clones. The term "isolated", as used herein in reference to a polypeptide, means a polypeptide encoded by an isolated nucleic acid sequence, as well as polypeptides synthesized by, for example, chemical synthetic methods, and polypeptides separated from biological materials, and then purified using conventional protein analytical or preparatory procedures. Nucleic acids which encode the foregoing polypeptides also are embraced by the invention. Preferably, a RUR-1 or RUR-1 antisense-encoded protein is at least 90%, more preferably 95%, and most preferably 99% identical to the amino acid sequence set forth in either SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:5.

A unique fragment of a RUR-1 or RUR-1 antisense-encoded protein, in general, has the features and characteristics of unique fragments as discussed above in connection with nucleic acids. As will be recognized by those skilled in the art, the size of the unique fragment will depend upon factors such as whether the fragment constitutes a portion of a conserved protein domain. Thus, some regions of SEQ ID NO:2 and SEQ ID NO:5 will require longer segments to be unique while others will require only short segments, typically between 5 and 12 amino acids (e.g. 5, 6, 7, 8, 9, 10, 11 and 12 amino acids long). Virtually any segment of SEQ ID NO:2 or SEQ ID NO:5 that is 10 or more amino acids in length will be unique.

Unique fragments of a polypeptide preferably are those fragments which retain a distinct functional capability of the polypeptide. Functional capabilities which can be retained in a unique fragment of a polypeptide include presentation by HLA molecules, interaction with antibodies, interaction with other polypeptides or fragments thereof, selective binding of nucleic acids, and enzymatic activity. A tumor rejection antigen is an example of a unique

fragment of a tumor associated polypeptide which retains the functional capability of HLA binding and interaction with cytotoxic T lymphocytes. Tumor rejection antigens presented by HLA class I molecules typically are 9 amino acids in length, although peptides of 8, 9 and 10 and more amino acids also retain the capability to interact with HLA and cytotoxic T lymphocyte to an extent effective to provoke a cytotoxic T lymphocyte response (see, e.g., Van den Eynde & Brichard, *Curr. Opin. Immunol.* 7:674-681, 1995; Coulie et al., *Stem Cells* 13:393-403, 1995). Similarly, tumor rejection antigens (e.g., 10 - 20 amino acids in length) can interact with HLA class II molecules and T helper lymphocytes, provoking proliferation and response of the T helper lymphocytes (see, e.g., Van den Eynde & van der Bruggen, *Curr. Opin. Immunol.* 9:684-693, 1997; Topalian et al., *J. Exp. Med.* 183:1965-1971, 1996).

Those skilled in the art are well versed in methods for selecting unique amino acid sequences, typically on the basis of the ability of the unique fragment to selectively distinguish the sequence of interest from non-RUR-1 or RUR-1 antisense-encoded family polypeptides. A comparison of the sequence of the fragment to those on known data bases typically is all that is necessary. Certain functional aspects of unique fragments of the RUR-1 or RUR-1 antisense-encoded polypeptides can be determined by employing well-known computer algorithms to compare RUR-1 sense-encoded or RUR-1 antisense-encoded polypeptide fragments to fragments of other polypeptides. For example, an HLA-peptide binding algorithm (available on the National Institutes of Health website [<http://bimas.dcrt.nih.gov>]; Parker et al., *J. Immunol.* 152:163, 1994) can be used to predict the HLA binding properties of peptides derived from the protein encoded by RUR-1 antisense cDNA. For example, the HLA-B7 binding score (half time of dissociation) of the RUR-1 antisense cDNA- encoded peptide (SEQ ID NO:3) is predicted to be 1200, which indicates a very stable interaction of the peptide with the HLA molecule.

The skilled artisan will also realize that conservative amino acid substitutions may be made in RUR-1 sense cDNA or RUR-1 antisense cDNA-encoded polypeptides to provide functional homologs or variants of the foregoing polypeptides, i.e, the homologs or variants , retain one or more of the functional capabilities of the RUR-1 sense cDNA or RUR-1 antisense cDNA-encoded polypeptides. As used herein, a "conservative amino acid substitution" refers to an amino acid substitution which does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. Conservative substitutions of amino acids include substitutions made amongst amino acids within the

following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

Functional homologs or variants of RUR-1 sense cDNA or RUR-1 antisense cDNA-encoded polypeptides can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, e.g. *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Exemplary functional variants of the RUR-1 sense cDNA or RUR-1 antisense cDNA-encoded polypeptides include conservative amino acid substitutions of SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:5. Conservative amino acid substitutions in the amino acid sequence of RUR-1 sense cDNA or RUR-1 antisense cDNA-encoded polypeptides to produce functional variants of such polypeptides typically are made by alteration of the nucleic acid encoding such polypeptides (SEQ ID NO:1, SEQ ID NO:4). Such substitutions can be made by a variety of methods known to one of ordinary skill in the art. For example, amino acid substitutions may be made by PCR-directed mutation, site-directed mutagenesis according to the method of Kunkel (Kunkel, *Proc. Nat. Acad. Sci. U.S.A.* 82: 488-492, 1985), or by chemical synthesis of a RUR-1 sense or antisense nucleic acid encoding a polypeptide. Where amino acid substitutions are made to a small unique fragment of a RUR-1 sense cDNA or RUR-1 antisense cDNA-encoded polypeptide, such as a 9 amino acid peptide, the substitutions can be made by directly synthesizing the peptide.

The activity of functional homologs or variants (including unique fragments) of RUR-1 sense cDNA or RUR-1 antisense cDNA-encoded polypeptides can be tested by cloning the gene encoding the altered polypeptide into a bacterial or mammalian expression vector, introducing the vector into an appropriate host cell, expressing the altered polypeptide, and testing for a functional capability of the polypeptides as disclosed herein.

For functional variants or homologs of RUR-1 antisense cDNA-encoded TRAs, the aforementioned computer algorithms can be used to predict amino acid substitutions which can be made to polypeptide sequences to prepare functional variants or homologs which retain HLA binding. For example, substitution of the W at position 4 of SEQ ID NO:3 with either a F or a Y residue (which are conservative amino acid substitutions) is predicted to have no effect on the binding of the TRA peptide to HLA-B7. In contrast, substitution of an H or K

residue for the R residue at position 3 of SEQ ID NO:3 is predicted to reduce HLA binding (increase dissociation) by 10-fold. Such variant TRA peptides then can be tested for biological activity as described in the Examples below.

As mentioned above, the invention embraces antisense oligonucleotides that selectively bind to a RUR-1 sense or RUR-1 antisense nucleic acid molecule encoding a protein, to decrease transcription and/or translation of RUR-1 sense or RUR-1 antisense nucleic acids. This is desirable in virtually any medical condition wherein a reduction in RUR-1 sense or RUR-1 antisense gene product expression is desirable, including to reduce any aspect of a tumor cell phenotype attributable to RUR-1 sense or RUR-1 antisense gene expression. Antisense molecules, in this manner, can be used to slow down or arrest such aspects of a tumor cell phenotype.

As used herein, the term "antisense oligonucleotide" or "antisense" describes an oligonucleotide that is an oligoribonucleotide, oligodeoxyribonucleotide, modified oligoribonucleotide, or modified oligodeoxyribonucleotide which hybridizes under physiological conditions to DNA comprising a particular gene or to an mRNA transcript of that gene and, thereby, inhibits the transcription of that gene and/or the translation of that mRNA. The antisense molecules are designed so as to interfere with transcription or translation of a target gene upon hybridization with the target gene. Those skilled in the art will recognize that the exact length of the antisense oligonucleotide and its degree of complementarity with its target will depend upon the specific target selected, including the sequence of the target and the particular bases which comprise that sequence. It is preferred that the antisense oligonucleotide be constructed and arranged so as to bind selectively with the target under physiological conditions, i.e., to hybridize substantially more to the target sequence than to any other sequence in the target cell under physiological conditions. Based upon SEQ ID NO:1 and/or SEQ ID NO:4, or upon allelic or homologous genomic and/or DNA sequences, one of skill in the art can easily choose and synthesize any of a number of appropriate antisense molecules for use in accordance with the present invention. In order to be sufficiently selective and potent for inhibition, such antisense oligonucleotides should comprise at least 7 (Wagner et al., *Nature Biotechnology* 14:840-844, 1996) and, more preferably, at least 15 consecutive bases which are complementary to the target. Most preferably, the antisense oligonucleotides comprise a complementary sequence of 20-30 bases. Although oligonucleotides may be chosen which are antisense to any region of the

gene or mRNA transcripts, in preferred embodiments the antisense oligonucleotides correspond to N-terminal or 5' upstream sites such as translation initiation, transcription initiation or promoter sites. In addition, 3'-untranslated regions may be targeted. Targeting to mRNA splicing sites has also been used in the art but may be less preferred if alternative mRNA splicing occurs. In addition, the antisense is targeted, preferably, to sites in which mRNA secondary structure is not expected (see, e.g., Sainio et al., *Cell Mol. Neurobiol.* 14(5):439-457, 1994) and at which proteins are not expected to bind. Finally, although SEQ ID NO:1 and SEQ ID NO:4 disclose cDNA sequences, one of ordinary skill in the art may easily derive the genomic DNA corresponding to these cDNAs, as is described in the Examples. Thus, the present invention also provides for antisense oligonucleotides which are complementary to the genomic DNA corresponding to SEQ ID NO:1 and SEQ ID NO:4. Similarly, antisense to allelic or homologous DNAs and genomic DNAs are enabled without undue experimentation.

In one set of embodiments, the antisense oligonucleotides of the invention may be composed of "natural" deoxyribonucleotides, ribonucleotides, or any combination thereof. That is, the 5' end of one native nucleotide and the 3' end of another native nucleotide may be covalently linked, as in natural systems, via a phosphodiester internucleoside linkage. These oligonucleotides may be prepared by art recognized methods which may be carried out manually or by an automated synthesizer. They also may be produced recombinantly by vectors.

In preferred embodiments, however, the antisense oligonucleotides of the invention also may include "modified" oligonucleotides. That is, the oligonucleotides may be modified in a number of ways which do not prevent them from hybridizing to their target but which enhance their stability or targeting or which otherwise enhance their therapeutic effectiveness.

The term "modified oligonucleotide" as used herein describes an oligonucleotide in which (1) at least two of its nucleotides are covalently linked via a synthetic internucleoside linkage (i.e., a linkage other than a phosphodiester linkage between the 5' end of one nucleotide and the 3' end of another nucleotide) and/or (2) a chemical group not normally associated with nucleic acids has been covalently attached to the oligonucleotide. Preferred synthetic internucleoside linkages are phosphorothioates, alkylphosphonates, phosphorodithioates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamides, peptides, and carboxymethyl esters.

The term "modified oligonucleotide" also encompasses oligonucleotides with a covalently modified base and/or sugar. For example, modified oligonucleotides include oligonucleotides having backbone sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Thus modified oligonucleotides may include a 2'-O-alkylated ribose group. In addition, modified oligonucleotides may include sugars such as arabinose instead of ribose. Modified oligonucleotides also can include base analogs such as C-5 propyne modified bases (Wagner et al., *Nature Biotechnology* 14:840- 844, 1996). The present invention, thus, contemplates pharmaceutical preparations containing modified antisense molecules that are complementary to and hybridizable with, under physiological conditions, RUR-1 sense or RUR-1 antisense nucleic acids encoding polypeptides, together with pharmaceutically acceptable carriers.

Antisense oligonucleotides may be administered as part of a pharmaceutical composition. Such a pharmaceutical composition may include the antisense oligonucleotides in combination with any standard pharmaceutically acceptable carriers which are known in the art. The compositions should be sterile and contain a therapeutically effective amount of the antisense oligonucleotides in a unit of weight or volume suitable for administration to a patient. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. The characteristics of the carrier will depend on the route of administration. Pharmaceutically acceptable carriers include diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials which are well known in the art.

It will also be seen from the examples that the invention embraces the use of the RUR-1 sense and/or RUR-1 antisense sequences in expression vectors, as well as to transfect host cells and cell lines, be these prokaryotic (e.g., *E. coli*), or eukaryotic (e.g., CHO cells, COS cells, yeast expression systems and recombinant baculovirus expression in insect cells). Especially useful are mammalian cells such as mouse, hamster, pig, goat, primate, etc. They may be of a wide variety of tissue types, including mast cells, fibroblasts, oocytes and lymphocytes, and they may be primary cells or cell lines. Specific examples include dendritic cells, U293 cells, peripheral blood leukocytes, bone marrow stem cells and embryonic stem cells. The expression vectors require that the pertinent sequence, i.e., those nucleic acids described supra, be operably linked to a promoter. As it is known that a human HLA class I

molecule presents a tumor rejection antigen encoded by the RUR-1 antisense nucleic acid molecule (i.e., SEQ ID NO:3), the expression vector may also include a nucleic acid sequence coding for the HLA molecule that presents any particular tumor rejection antigen derived from these genes and polypeptides (HLA-B7 for the SEQ ID NO:3 TRA). Alternatively, the nucleic acid sequence coding for such a HLA molecule can be contained within a separate expression vector. In a situation where the vector contains both coding sequences, the single vector can be used to transfect a cell which does not normally express either one. Where the coding sequences for the tumor rejection antigen precursor and the HLA molecule which presents it are contained on separate expression vectors, the expression vectors can be cotransfected. The tumor rejection antigen precursor coding sequence may be used alone, when, e.g. the host cell already expresses a HLA molecule which presents a RUR-1 antisense encoded TRA. Of course, there is no limit on the particular host cell which can be used. As the vectors which contain the two coding sequences may be used in any antigen-presenting cells if desired, and the gene for tumor rejection antigen precursor can be used in host cells which do not express a HLA molecule which presents a RUR-1 antisense encoded TRA. Further, cell-free transcription systems may be used in lieu of cells.

The skilled artisan can determine which HLA molecule binds to tumor rejection antigens derived from tumor rejection antigen precursors by, e.g., experiments utilizing antibodies to block specifically individual HLA class I molecules. For example, as shown in the Examples, antibodies which bind selectively to HLA-B7 prevented efficient presentation of the TRA set forth in SEQ ID NO:3. Thus, the binding specificity of additional TRAs derived from the RUR-1 antisense cDNA-encoded polypeptide can be determined by similar experiments using widely available anti-HLA antibodies.

As used herein, a "vector" may be any of a number of nucleic acids into which a desired sequence may be inserted by restriction and ligation for transport between different genetic environments or for expression in a host cell. Vectors are typically composed of DNA although RNA vectors are also available. Vectors include, but are not limited to, plasmids and phagemids. A cloning vector is one which is able to replicate in a host cell, and which is further characterized by one or more endonuclease restriction sites at which the vector may be cut in a determinable fashion and into which a desired DNA sequence may be ligated such that the new recombinant vector retains its ability to replicate in the host cell. In the case of plasmids, replication of the desired sequence may occur many times as the plasmid increases

in copy number within the host bacterium or just a single time per host before the host reproduces by mitosis. In the case of phage, replication may occur actively during a lytic phase or passively during a lysogenic phase. An expression vector is one into which a desired DNA sequence may be inserted by restriction and ligation such that it is operably joined to regulatory sequences and may be expressed as an RNA transcript. Vectors may further contain one or more marker sequences suitable for use in the identification of cells which have or have not been transformed or transfected with the vector. Markers include, for example, genes encoding proteins which increase or decrease either resistance or sensitivity to antibiotics or other compounds, genes which encode enzymes whose activities are detectable by standard assays known in the art (e.g. β -galactosidase or alkaline phosphatase), and genes which visibly affect the phenotype of transformed or transfected cells, hosts, colonies or plaques (e.g. green fluorescent protein). Preferred vectors are those capable of autonomous replication and expression of the structural gene products present in the DNA segments to which they are operably joined.

As used herein, a coding sequence and regulatory sequences are said to be "operably" joined when they are covalently linked in such a way as to place the expression or transcription of the coding sequence under the influence or control of the regulatory sequences. If it is desired that the coding sequences be translated into a functional protein, two DNA sequences are said to be operably joined if induction of a promoter in the 5' regulatory sequences results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequences, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a promoter region would be operably joined to a coding sequence if the promoter region were capable of effecting transcription of that DNA sequence such that the resulting transcript might be translated into the desired protein or polypeptide.

The precise nature of the regulatory sequences needed for gene expression may vary between species or cell types, but shall in general include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of transcription and translation respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing regulatory sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined gene.

Regulatory sequences may also include enhancer sequences or upstream activator sequences as desired. The vectors of the invention may optionally include 5' leader or signal sequences, 5' or 3'. The choice and design of an appropriate vector is within the ability and discretion of one of ordinary skill in the art.

5 Expression vectors containing all the necessary elements for expression are commercially available and known to those skilled in the art. See *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. Cells are genetically engineered by the introduction into the cells of heterologous DNA (RNA) encoding the RUR-1 sense-encoded
10 or RUR-1 antisense-encoded polypeptide or fragment or variant thereof. That heterologous DNA (RNA) is placed under operable control of transcriptional elements to permit the expression of the heterologous DNA in the host cell.

Preferred systems for mRNA expression in mammalian cells are those such as pRc/CMV (available from Invitrogen, San Diego, CA) that contain a selectable marker such
15 as a gene that confers G418 resistance (which facilitates the selection of stably transfected cell lines) and the human cytomegalovirus (CMV) enhancer-promoter sequences. Additionally, suitable for expression in primate or canine cell lines is the pCEP4 vector (Invitrogen), which contains an Epstein Barr virus (EBV) origin of replication, facilitating the maintenance of plasmid as a multicopy extrachromosomal element. Another expression vector is the pEF-
20 BOS plasmid containing the promoter of polypeptide Elongation Factor 1 α , which stimulates efficiently transcription *in vitro*. The plasmid is described by Mishizuma and Nagata (*Nuc. Acids Res.* 18:5322, 1990), and its use in transfection experiments is disclosed by, for example, Demoulin (*Mol. Cell. Biol.* 16:4710-4716, 1996). Still another preferred expression vector is an adenovirus, described by Stratford-Perricaudet, which is defective for E1 and E3
25 proteins (*J. Clin. Invest.* 90:626-630, 1992). The use of the adenovirus as an Adeno.P1A recombinant is disclosed by Warnier et al., in intradermal injection in mice for immunization against P1A (*Int. J. Cancer*, 67:303-310, 1996).

The invention also embraces so-called expression kits, which allow the artisan to prepare a desired expression vector or vectors. Such expression kits include at least separate
30 portions of each of the previously discussed coding sequences. Other components may be added, as desired, as long as the previously mentioned sequences, which are required, are included.

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The invention also involves agents which bind to RUR-1 sense-encoded and/or RUR-1 antisense-encoded polypeptides and in certain embodiments preferably to unique fragments of those polypeptides. Such binding partners can be used in screening assays to detect the presence or absence of the RUR-1 sense-encoded and/or RUR-1 antisense-encoded polypeptide and in purification protocols to isolate such polypeptides. Likewise, such binding partners can be used to selectively target drugs, toxins or other molecules to tumor cells which present RUR-1 antisense-encoded tumor associated polypeptides. In this manner, tumor cells which express RUR-1 antisense-encoded polypeptides can be treated with cytotoxic compounds.

The invention, therefore, involves antibodies or fragments of antibodies having the ability to selectively bind to RUR-1 sense-encoded and/or RUR-1 antisense-encoded polypeptides, and preferably to unique fragments thereof. Antibodies include polyclonal and monoclonal antibodies, prepared according to conventional methodology.

Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W.R. (1986) The Experimental Foundations of Modern Immunology Wiley & Sons, Inc., New York; Roitt, I. (1991) Essential Immunology, 7th Ed., Blackwell Scientific Publications, Oxford). The pFc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an F(ab')₂ fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and

the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDR3). The CDRs, and in particular the CDR3 regions, and more particularly the heavy chain CDR3, are largely responsible for antibody specificity.

5 It is now well-established in the art that the non-CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody.

10 See, e.g., U.S. patents 4,816,567, 5,225,539, 5,585,089, 5,693,762 and 5,859,205.

Thus, for example, PCT International Publication Number WO 92/04381 teaches the production and use of humanized murine RSV antibodies in which at least a portion of the murine FR regions have been replaced by FR regions of human origin. Such antibodies, including fragments of intact antibodies with antigen-binding ability, are often referred to as
15 "chimeric" antibodies.

Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for F(ab')₂, Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by
20 homologous human or non-human sequences; chimeric F(ab')₂ fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which
25 the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous human or non-human sequences. The present invention also includes so-called single chain antibodies. Thus, the invention involves polypeptides of numerous size and type that bind specifically to RUR-1 sense-encoded and/or RUR-1 antisense-encoded polypeptides. These polypeptides may be derived also from sources other than antibody technology. For example, such polypeptide binding agents can be provided by degenerate peptide libraries which can be
30 readily prepared in solution, in immobilized form or as phage display libraries. Combinatorial libraries also can be synthesized of peptides containing one or more amino acids. Libraries further can be synthesized of peptoids and non-peptide synthetic moieties.

Phage display can be particularly effective in identifying binding peptides useful according to the invention. Briefly, one prepares a phage library (using e.g. m13, fd, or lambda phage), displaying inserts from 4 to about 80 amino acid residues using conventional procedures. The inserts may represent a completely degenerate or biased array. One then can
5 select phage-bearing inserts which bind to a RUR-1 sense-encoded or RUR-1 antisense-encoded polypeptide. This process can be repeated through several cycles of reselection of phage that bind to the RUR-1 sense-encoded or RUR-1 antisense-encoded polypeptide. Repeated rounds lead to enrichment of phage bearing particular sequences. DNA sequence analysis can be conducted to identify the sequences of the expressed polypeptides. The
10 minimal linear portion of the sequence that binds to the RUR-1 sense-encoded or RUR-1 antisense-encoded polypeptide can be determined. One can repeat the procedure using a biased library containing inserts containing part or all of the minimal linear portion plus one or more additional degenerate residues upstream or downstream thereof. Thus, the RUR-1 sense-encoded or RUR-1 antisense-encoded polypeptides of the invention can be used to
15 screen peptide libraries, including phage display libraries, to identify and select peptide binding partners of the RUR-1 sense-encoded or RUR-1 antisense-encoded polypeptides of the invention. Such molecules can be used, as described, for screening assays, for diagnostic assays, for purification protocols or for targeting drugs, toxins and/or labeling agents (e.g. radioisotopes, fluorescent molecules, etc.) to cells which present RUR-1 antisense-encoded
20 peptides on the cell surface. Such binding agent molecules can also be prepared to bind complexes of a RUR-1 antisense-encoded polypeptide and an HLA molecule by selecting the binding agent using such complexes. Drug molecules that would disable or destroy tumor cells which express such complexes or RUR-1 antisense-encoded polypeptides are known to those skilled in the art and are commercially available. For example, the immunotoxin art
25 provides examples of toxins which are effective when delivered to a cell by an antibody or fragment thereof. Examples of toxins include ribosome-damaging toxins derived from plants or bacterial such as ricin, abrin, saporin, *Pseudomonas* endotoxin, diphtheria toxin, A chain toxins, blocked ricin, etc.

The invention as described herein has a number of uses, some of which are described
30 herein. First, the invention permits the artisan to diagnose a disorder characterized by expression of the TRAP. These methods involve determining expression of the TRAP gene, and/or TRAs derived therefrom. In the former situation, such determinations can be carried

out via any standard nucleic acid determination assay, including the polymerase chain reaction as exemplified in the examples below, or assaying with labeled hybridization probes.

In general, methods for diagnosing a disorder that is characterized by expression of a tumor associated nucleic acid or polypeptide involve contacting a biological sample isolated from a subject with an agent specific for the tumor associated nucleic acid or polypeptide to detect the presence of the tumor associated nucleic acid or polypeptide in the biological sample. As used herein, "contacting" means placing the biological sample in sufficient proximity to the agent and under the appropriate conditions of, e.g., concentration, temperature, time, ionic strength, to allow the specific interaction between the agent and tumor associated nucleic acid or polypeptide that are present in the biological sample. In general, the conditions for contacting the agent with the biological sample are conditions known by those of ordinary skill in the art to facilitate a specific interaction between a molecule and its cognate (e.g., a protein and its receptor cognate, an antibody and its protein antigen cognate, a nucleic acid and its complementary sequence cognate) in a biological sample. Exemplary conditions for facilitating a specific interaction between a molecule and its cognate are described in U.S. Patent No. 5,108,921, issued to Low et al.

The biological sample can be located *in vivo* or *in vitro*. For example, the biological sample can be a tissue *in vivo* and the agent specific for the tumor associated nucleic acid or polypeptide can be used to detect the presence of such molecules in the tissue (e.g., for imaging portions of the tissue that express the tumor associated gene products). Alternatively, the biological sample can be located *in vitro* (e.g., a blood sample, tumor biopsy, tissue extract). In a particularly preferred embodiment, the biological sample can be a cell-containing sample, more preferably a sample containing tumor cells. Preferably the biological samples are not derived from liver, kidney, bladder or testis.

The foregoing methods of diagnosis can be used in the determination of the subject's prognosis, e.g., the progression or regression of a condition characterized by RUR-1 antisense cDNA expression. Further, the diagnostic methods described herein provide a means to follow the effect of treatments (e.g. vaccines, CTLs) on a tumor or other RUR-1 related condition.

Methods of treatment of disorders characterized by expression of a tumor associated nucleic acid molecule or expression product thereof also are provided. In general, methods of treatment include administering to the subject an amount of an agent which enriches

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selectively in the subject the presence of complexes of a HLA molecule and a tumor rejection antigen derived from a RUR-1 antisense-encoded tumor associated polypeptide. The agent is administered in an amount effective to ameliorate the disorder. Agents for treatment of disorders characterized by expression of RUR-1 antisense nucleic acids or encoded polypeptides include polypeptides comprising the amino acid sequence of SEQ ID NO:3, and immunogenic fragments thereof, as well as autologous cytotoxic T cells specific for complexes of an HLA molecule and the foregoing tumor associated polypeptides and/or fragments or cells which express the foregoing tumor associated polypeptides and/or fragments and a HLA molecule.

The isolation of these genes also makes it possible to isolate the encoded polypeptide molecules, such as SEQ ID NO:2 and SEQ ID NO:5. A variety of methodologies well-known to the skilled practitioner can be utilized to obtain isolated polypeptide molecules. The protein may be purified from cells which naturally produce the protein. Alternatively, an expression vector may be introduced into cells to cause production of the protein. In another method, mRNA transcripts may be microinjected or otherwise introduced into cells to cause production of the encoded protein. Translation of mRNA in cell-free extracts such as the reticulocyte lysate system also may be used to produce protein. Those skilled in the art also can readily follow known methods for isolating proteins in order to obtain isolated RUR-1 sense-encoded or RUR-1 antisense-encoded polypeptides. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography and immune-affinity chromatography.

These isolated polypeptide molecules when processed and presented as the TRA (e.g., SEQ ID NO:3), or as complexes of TRA and HLA, such as HLA-B7, may be combined with materials such as adjuvants to produce vaccines useful in treating disorders characterized by expression of the TRAP molecule. In addition to RUR-1 antisense-encoded peptides, nucleic acids which encode peptide epitopes can be used to prepare vaccines. Preparation of nucleic acids and/or peptides for use in vaccines is well known in the art. When "disorder" is used herein, it refers to any pathological condition where the tumor rejection antigen precursor is expressed. An example of such a disorder is cancer, including renal cell carcinoma, colorectal carcinoma, melanoma, sarcoma, leukemia, etc..

In addition, vaccines can be prepared from cells which present the TRA/HLA complexes on their surface, such as non-proliferative cancer cells, non-proliferative

transfectants, etcetera. In all cases where cells are used as a vaccine, these can be cells transfected with coding sequences for one or both of the components necessary to provoke a CTL response, or be cells which already express both molecules without the need for transfection.

5 Therapeutic approaches based upon the disclosure are premised on a response by a subject's immune system, leading to lysis of TRA presenting cells, such as HLA-B7 cells. One such approach is the administration of autologous CTLs specific to the complex to a subject with abnormal cells of the phenotype at issue. It is within the skill of the artisan to develop such CTLs *in vitro*. Generally, a sample of cells taken from a subject, such as blood
10 cells, are contacted with a cell presenting the complex and capable of provoking CTLs to proliferate. The target cell can be a transfectant, such as a COS cell of the type described herein. These transfectants present the desired complex of their surface and, when combined with a CTL of interest, stimulate its proliferation. COS cells, such as those used herein are widely available, as are other suitable host cells. Specific production of a CTL is well known
15 to one of ordinary skill in the art. The clonally expanded autologous CTLs then are administered to the subject. Other CTLs specific to RUR-1 antisense-encoded TRAs may be isolated and administered by similar methods.

 Another method for selecting antigen-specific CTL clones has recently been described (Altman et al., *Science* 274:94-96, 1996; Dunbar et al., *Curr. Biol.* 8:413-416, 1998), in which
20 fluorogenic tetramers of MHC class I molecule/peptide complexes are used to detect specific CTL clones. Briefly, soluble MHC class I molecules are folded *in vitro* in the presence of β_2 -microglobulin and a peptide antigen which binds the class I molecule. After purification, the MHC/peptide complex is purified and labeled with biotin. Tetramers are formed by mixing the biotinylated peptide-MHC complex with labeled avidin (e.g. phycoerythrin) at a molar
25 ratio of 4:1. Tetramers are then contacted with a source of CTLs such as peripheral blood or lymph node. The tetramers bind CTLs which recognize the peptide antigen/MHC class I complex. Cells bound by the tetramers can be sorted by fluorescence activated cell sorting to isolate the reactive CTLs. The isolated CTLs then can be expanded *in vitro* for use as described herein.

30 To detail a therapeutic methodology, referred to as adoptive transfer (Greenberg, *J. Immunol.* 136(5): 1917, 1986; Riddel et al., *Science* 257: 238, 1992; Lynch et al, *Eur. J. Immunol.* 21: 1403-1410, 1991; Kast et al., *Cell* 59: 603-614, 1989), cells presenting the

desired complex are combined with CTLs leading to proliferation of the CTLs specific thereto. The proliferated CTLs are then administered to a subject with a cellular abnormality which is characterized by certain of the abnormal cells presenting the particular complex. The CTLs then lyse the abnormal cells, thereby achieving the desired therapeutic goal.

5 The foregoing therapy assumes that at least some of the subject's abnormal cells present the relevant HLA/TRA complex. This can be determined very easily, as the art is very familiar with methods for identifying cells which present a particular HLA molecule, as well as how to identify cells expressing DNA of the pertinent sequences, for example a RUR-1 antisense sequence. Once cells presenting the relevant complex are identified via the
10 foregoing screening methodology, they can be combined with a sample from a patient, where the sample contains CTLs. If the complex presenting cells are lysed by the mixed CTL sample, then it can be assumed that a RUR-1 antisense-encoded TRA is being presented, and the subject is an appropriate candidate for the therapeutic approaches set forth *supra*.

 Adoptive transfer is not the only form of therapy that is available in accordance with
15 the invention. CTLs can also be provoked *in vivo*, using a number of approaches. One approach is the use of non-proliferative cells expressing the complex. The cells used in this approach may be those that normally express the complex, such as irradiated tumor cells or cells transfected with one or both of the genes necessary for presentation of the complex. Chen et al., *Proc. Natl. Acad. Sci. USA* 88: 110-114 (1991) exemplifies this approach,
20 showing the use of transfected cells expressing HPV E7 peptides in a therapeutic regime. Various cell types may be used. Similarly, vectors carrying one or both of the genes of interest may be used. Viral or bacterial vectors are especially preferred. For example, nucleic acids which encode SEQ ID NO:3 may be operably linked to promoter and enhancer sequences which direct expression of the TRA in certain tissues or cell types. The nucleic
25 acid may be incorporated into an expression vector. Expression vectors may be unmodified extrachromosomal nucleic acids, plasmids or viral genomes constructed or modified to enable insertion of exogenous nucleic acids, such as those encoding SEQ ID NO:3. Nucleic acids encoding a RUR-1 antisense-encoded TRA also may be inserted into a retroviral genome, thereby facilitating integration of the nucleic acid into the genome of the target tissue or cell
30 type. In these systems, the gene of interest is carried by a microorganism, e.g., a Vaccinia virus, retrovirus or the bacteria BCG, and the materials *de facto* "infect" host cells. The cells which result present the complex of interest, and are recognized by autologous CTLs, which

then proliferate.

A similar effect can be achieved by combining a TRAP or a stimulatory fragment thereof with an adjuvant to facilitate incorporation into HLA presenting cells *in vivo*. The TRAP is processed to yield the peptide partner of the HLA molecule while the TRA is presented without the need for further processing. Generally, subjects can receive an intradermal injection of an effective amount of TRAP, and/or TRAs derived therefrom. Initial doses can be followed by booster doses, following immunization protocols standard in the art.

Yet another approach which can be utilized to provoke an immune response is to provide TRAs in the form of a nucleic acid encoding a series of epitopes, known as "polytopes". The epitopes can be arranged in sequential or overlapping fashion (*see, e.g.*, Thomson et al., *Proc. Natl. Acad. Sci. USA* 92:5845-5849, 1995; Gilbert et al., *Nature Biotechnol.* 15:1280-1284, 1997), with or without the natural flanking sequences, and can be separated by unrelated linker sequences if desired. The polytope is processed to generate individual epitopes which are recognized by the immune system for generation of immune responses.

Thus, for example, peptides derived from the polypeptide having an amino acid sequence encoded by the nucleic acid of SEQ ID NO:1, and which are presented by MHC molecules and recognized by CTL or T helper lymphocytes can be combined with peptides from other tumor rejection antigens (e.g. by preparation of hybrid nucleic acids or polypeptides) to form polytopes. Exemplary tumor associated peptide antigens (which are presented by MHC class I or II) that can be administered to induce or enhance an immune response are derived from tumor associated genes and encoded proteins including MAGE-1, MAGE-2, MAGE-3, MAGE-4, MAGE-5, MAGE-6, MAGE-7, MAGE-8, MAGE-9, MAGE-10, MAGE-11, MAGE-12, MAGE-13, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, BAGE-1, RAGE-1, LB33/MUM-1, PRAME, NAG, MAGE-B2, MAGE-B3, MAGE-B4, tyrosinase, brain glycogen phosphorylase, Melan-A, MAGE-C1, MAGE-C2, NY-ESO-1, LAGE-1, SSX-1, SSX-2 (HOM-MEL-40), SSX-4, SSX-5, SCP-1 and CT-7. See, for example, PCT application publication no. WO96/10577. Other examples will be known to one of ordinary skill in the art (for example, see Coulie, *Stem Cells* 13:393-403, 1995), and can be used in the invention in a like manner as those disclosed herein. One of ordinary skill in the art can prepare polypeptides comprising one or more RUR-1 antisense-encoded peptides and one or more of the foregoing tumor rejection antigen peptides, or

nucleic acids encoding such polypeptides, according to standard procedures of molecular biology.

Thus polytopes are groups of two or more potentially immunogenic or immune response stimulating peptides which can be joined together in various arrangements (e.g. concatenated, overlapping). The polytope (or nucleic acid encoding the polytope) can be administered in a standard immunization protocol, e.g. to animals, to test the effectiveness of the polytope in stimulating, enhancing and/or provoking an immune response.

The peptides can be joined together directly or via the use of flanking sequences to form polytopes, and the use of polytopes as vaccines is well known in the art (see, e.g., Thomson et al., *Proc. Acad. Natl. Acad. Sci USA* 92(13):5845-5849, 1995; Gilbert et al., *Nature Biotechnol.* 15(12):1280-1284, 1997; Thomson et al., *J. Immunol.* 157(2):822-826, 1996; Tam et al., *J. Exp. Med.* 171(1):299-306, 1990). For example, Tam showed that polytopes consisting of both MHC class I and class II binding epitopes successfully generated antibody and protective immunity in a mouse model. Tam also demonstrated that polytopes comprising "strings" of epitopes are processed to yield individual epitopes which are presented by MHC molecules and recognized by CTLs. Thus polytopes containing various numbers and combinations of epitopes can be prepared and tested for recognition by CTLs and for efficacy in increasing an immune response.

It is known that tumors express a set of tumor antigens, of which only certain subsets may be expressed in the tumor of any given patient. Polytopes can be prepared which correspond to the different combination of epitopes representing the subset of tumor rejection antigens expressed in a particular patient. Polytopes also can be prepared to reflect a broader spectrum of tumor rejection antigens known to be expressed by a tumor type. Polytopes can be introduced to a patient in need of such treatment as polypeptide structures, or via the use of nucleic acid delivery systems known in the art (see, e.g., Allsopp et al., *Eur. J. Immunol.* 26(8):1951-1959, 1996). Adenovirus, pox virus, Ty-virus like particles, adeno-associated virus, plasmids, bacteria, etc. can be used in such delivery. One can test the polytope delivery systems in mouse models to determine efficacy of the delivery system. The systems also can be tested in human clinical trials.

As part of the immunization compositions, one or more tumor antigens or stimulatory fragments thereof are administered with one or more adjuvants to induce an immune response or to increase an immune response. An adjuvant is a substance incorporated into or

administered with antigen which potentiates the immune response. Adjuvants may enhance the immunological response by providing a reservoir of antigen (extracellularly or within macrophages), activating macrophages and stimulating specific sets of lymphocytes.

Adjuvants of many kinds are well known in the art. Specific examples of adjuvants include monophosphoryl lipid A (MPL, SmithKline Beecham), a congener obtained after purification and acid hydrolysis of *Salmonella minnesota* Re 595 lipopolysaccharide; saponins including QS21 (SmithKline Beecham), a pure QA-21 saponin purified from *Quillja saponaria* extract; DQS21, described in PCT application WO96/33739 (SmithKline Beecham); QS-7, QS-17, QS-18, and QS-L1 (So et al., *Mol. Cells* 7:178-186, 1997); incomplete Freund's adjuvant; complete Freund's adjuvant; montanide; and various water-in-oil emulsions prepared from biodegradable oils such as squalene and/or tocopherol. Preferably, the peptides are administered mixed with a combination of DQS21/MPL. The ratio of DQS21 to MPL typically will be about 1:10 to 10:1, preferably about 1:5 to 5:1 and more preferably about 1:1. Typically for human administration, DQS21 and MPL will be present in a vaccine formulation in the range of about 1 µg to about 100 µg. Other adjuvants are known in the art and can be used in the invention (see, e.g. Goding, *Monoclonal Antibodies: Principles and Practice*, 2nd Ed., 1986). Methods for the preparation of mixtures or emulsions of peptide and adjuvant are well known to those of skill in the art of vaccination.

Other agents which stimulate the immune response of the subject can also be administered to the subject. For example, other cytokines are also useful in vaccination protocols as a result of their lymphocyte regulatory properties. Many other cytokines useful for such purposes will be known to one of ordinary skill in the art, including interleukin-12 (IL-12) which has been shown to enhance the protective effects of vaccines (see, e.g., *Science* 268: 1432-1434, 1995), GM-CSF and IL-18. Thus cytokines can be administered in conjunction with antigens and adjuvants to increase the immune response to the antigens.

There are a number of immune response potentiating compounds that can be used in vaccination protocols. These include costimulatory molecules provided in either protein or nucleic acid form. Such costimulatory molecules include the B7-1 and B7-2 (CD80 and CD86 respectively) molecules which are expressed on dendritic cells (DC) and interact with the CD28 molecule expressed on the T cell. This interaction provides costimulation (signal 2) to an antigen/MHC/TCR stimulated (signal 1) T cell, increasing T cell proliferation and effector function. B7 also interacts with CTLA4 (CD152) on T cells and studies involving CTLA4

and B7 ligands indicate that the B7-CTLA4 interaction can enhance antitumor immunity and CTL proliferation (Zheng P., et al. *Proc. Natl. Acad. Sci. USA* 95 (11):6284-6289 (1998)).

B7 typically is not expressed on tumor cells so they are not efficient antigen presenting cells (APCs) for T cells. Induction of B7 expression would enable the tumor cells to stimulate more efficiently CTL proliferation and effector function. A combination of B7/IL-6/IL-12 costimulation has been shown to induce IFN-gamma and a Th1 cytokine profile in the T cell population leading to further enhanced T cell activity (Gajewski et al., *J. Immunol.*, 154:5637-5648 (1995)). Tumor cell transfection with B7 has been discussed in relation to *in vitro* CTL expansion for adoptive transfer immunotherapy by Wang et al., (*J. Immunol.*, 19:1-8 (1986)).

Other delivery mechanisms for the B7 molecule would include nucleic acid (naked DNA) immunization (Kim J., et al. *Nat Biotechnol.*, 15:7:641-646 (1997)) and recombinant viruses such as adeno and pox (Wendtner et al., *Gene Ther.*, 4:7:726-735 (1997)). These systems are all amenable to the construction and use of expression cassettes for the coexpression of B7 with other molecules of choice such as the antigens or fragment(s) of antigens discussed herein (including polytopes) or cytokines. These delivery systems can be used for induction of the appropriate molecules *in vitro* and for *in vivo* vaccination situations. The use of anti-CD28 antibodies to directly stimulate T cells *in vitro* and *in vivo* could also be considered. Similarly, the inducible co-stimulatory molecule ICOS which induces T cell responses to foreign antigen could be modulated, for example, by use of anti-ICOS antibodies (Hutloff et al., *Nature* 397:263-266, 1999).

Lymphocyte function associated antigen-3 (LFA-3) is expressed on APCs and some tumor cells and interacts with CD2 expressed on T cells. This interaction induces T cell IL-2 and IFN-gamma production and can thus complement but not substitute, the B7/CD28 costimulatory interaction (Parra et al., *J. Immunol.*, 158:637-642 (1997), Fenton et al., *J. Immunother.*, 21:2:95-108 (1998)).

Lymphocyte function associated antigen-1 (LFA-1) is expressed on leukocytes and interacts with ICAM-1 expressed on APCs and some tumor cells. This interaction induces T cell IL-2 and IFN-gamma production and can thus complement but not substitute, the B7/CD28 costimulatory interaction (Fenton et al., *J. Immunother.*, 21:2:95-108 (1998)). LFA-1 is thus a further example of a costimulatory molecule that could be provided in a vaccination protocol in the various ways discussed above for B7.

Complete CTL activation and effector function requires Th cell help through the

interaction between the Th cell CD40L (CD40 ligand) molecule and the CD40 molecule expressed by DCs (Ridge et al., *Nature*, 393:474 (1998), Bennett et al., *Nature*, 393:478 (1998), Schoenberger et al., *Nature*, 393:480 (1998)). This mechanism of this costimulatory signal is likely to involve upregulation of B7 and associated IL-6/IL-12 production by the DC (APC). The CD40-CD40L interaction thus complements the signal 1 (antigen/MHC-TCR) and signal 2 (B7-CD28) interactions.

The use of anti-CD40 antibodies to stimulate DC cells directly would be expected to enhance a response to tumor antigens which are normally encountered outside of a inflammatory context or are presented by non-professional APCs (tumor cells). In these situations Th help and B7 costimulation signals are not provided. This mechanism might be used in the context of antigen pulsed DC based therapies or in situations where Th epitopes have not been defined within known TRA precursors. Other methods for inducing maturation of dendritic cells, e.g., by increasing CD40-CD40L interaction, or by contacting DCs with CpG-containing oligodeoxynucleotides or stimulatory sugar moieties from extracellular matrix, are known in the art.

When administered, the therapeutic compositions of the present invention are administered in pharmaceutically acceptable preparations. Such preparations may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents.

The therapeutics of the invention can be administered by any conventional route, including injection or by gradual infusion over time. The administration may, for example, be oral, intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous, or transdermal. When antibodies are used therapeutically, a preferred route of administration is by pulmonary aerosol. Techniques for preparing aerosol delivery systems containing antibodies are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the antibodies, such as the paratope binding capacity (see, for example, Sciarra and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712). Those of skill in the art can readily determine the various parameters and conditions for producing antibody aerosols without resort to undue experimentation. When using antisense preparations of the invention, slow intravenous administration is preferred.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

The invention also contemplates delivery of nucleic acids, polypeptides or peptides for vaccination. Delivery of polypeptides and peptides can be accomplished according to standard vaccination protocols which are well known in the art. In another embodiment, the delivery of nucleic acid is accomplished by *ex vivo* methods, i.e. by removing a cell from a subject, genetically engineering the cell to include a tumor antigen, and reintroducing the engineered cell into the subject. One example of such a procedure is outlined in U.S. Patent 5,399,346 and in exhibits submitted in the file history of that patent, all of which are publicly available documents. In general, it involves introduction *in vitro* of a functional copy of a gene into a cell(s) of a subject which contains a defective copy of the gene, and returning the genetically engineered cell(s) to the subject. The functional copy of the gene is under operable control of regulatory elements which permit expression of the gene in the genetically engineered cell(s). Numerous transfection and transduction techniques as well as appropriate expression vectors are well known to those of ordinary skill in the art, some of which are described in PCT application WO95/00654. *In vivo* gene therapy using plasmids or viral vectors such as adenovirus, adeno-associated virus, vaccinia virus, and the like also is contemplated according to the invention.

The preparations of the invention are administered in effective amounts. An effective amount is that amount of a pharmaceutical preparation that alone, or together with further doses, stimulates the desired response. In the case of treating cancer, the desired response is inhibiting the progression of the cancer. This may involve only slowing the progression of the disease temporarily, although more preferably, it involves halting the progression of the disease permanently. This can be monitored by routine methods or can be monitored

according to diagnostic methods of the invention discussed herein. The desired response to treatment of the disease or condition also can be delaying the onset or even preventing the onset of the disease or condition.

Such amounts will depend, of course, on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

Where it is desired to stimulate an immune response using a therapeutic composition of the invention, this may involve the stimulation of a humoral antibody response resulting in an increase in antibody titer in serum, a clonal expansion of cytotoxic lymphocytes, or some other desirable immunologic response. It is believed that doses of immunogens ranging from one nanogram/kilogram to 100 milligrams/kilogram, depending upon the mode of administration, would be effective. The preferred range is believed to be between 500 nanograms and 500 micrograms per kilogram. The absolute amount will depend upon a variety of factors, including the material selected for administration, whether the administration is in single or multiple doses, and individual patient parameters including those described above. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation.

Where antigenic peptides are used for vaccination, modes of administration which effectively deliver the tumor antigen and adjuvant, such that an immune response to the antigen is increased, can be used. For administration of a tumor antigen peptide in adjuvant, preferred methods include intradermal, intravenous, intramuscular and subcutaneous administration. Although these are preferred embodiments, the invention is not limited by the particular modes of administration disclosed herein. Standard references in the art (e.g., *Remington's Pharmaceutical Sciences*, 18th edition, 1990) provide modes of administration and formulations for delivery of immunogens with adjuvant or in a non-adjuvant carrier.

When administered, the pharmaceutical preparations of the invention are applied in pharmaceutically-acceptable amounts and in pharmaceutically-acceptable compositions. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. Such preparations may
5 routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not
10 limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

A tumor antigen composition may be combined, if desired, with a pharmaceutically-
15 acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being
20 co-mingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

The pharmaceutical compositions may contain suitable buffering agents, including: acetic acid in a salt; citric acid in a salt; boric acid in a salt; and phosphoric acid in a salt.

25 The pharmaceutical compositions also may contain, optionally, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal.

The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes
30 one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the active compound. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

5 Compositions suitable for parenteral administration conveniently comprise a sterile aqueous or non-aqueous preparation of tumor antigen polypeptides or nucleic acids, which is preferably isotonic with the blood of the recipient. This preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or
10 suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or di-glycerides. In addition, fatty acids
15 such as oleic acid may be used in the preparation of injectables. Carrier formulation suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA.

Examples

20 Example 1: Isolation of a sequence specifically expressed by renal cell carcinoma line LB9211-RCC.

Renal cell carcinoma line LE9211-RCC expresses several antigens recognized by autologous CTL (Brouwenstijn et al., *Int. J. Cancer* 68:177-182, 1996). It was previously found that one of the antigens was encoded by the *RAGE* gene (Gaugler et al.,
25 *Immunogenetics* 44:323-330, 1996). Another antigen has now been characterized which is recognized by a specific cytotoxic T lymphocyte clone, CTL 361A/21.

To generate CTLs, peripheral blood mononuclear cells from patient LE9211 were contacted with the LE9211-RCC cells. After 14 days, the mixture was observed for lysis of the carcinoma cells, which indicated that CTLs specific for a complex of peptide and HLA
30 molecule presented by the carcinoma cells were present in the sample. The lysis assay employed was a chromium release assay (Herin et al., *Int. J. Cancer* 39:390-396, 1987). Those mononuclear blood samples which showed high CTL activity were expanded and

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cloned via limiting dilution, and were screened again, using the same methodology. The CTL 361A/21 clone was then isolated.

Figure 1 shows that CTL 361A/21 lysed autologous tumor cell line LE9211-RCC, but not control LE9211-EBV cells (an autologous EBV-transformed B cell line) or NK-target K562 cells. Chromium release was measured after 4 hours. NK - target K562 cells are available from the ATCC, Rockville, Maryland.

To identify the HLA molecule that presented the antigen to CTL 361A/21, inhibition experiments were carried out where the production of TNF was tested in the presence of monoclonal antibodies directed against HLA molecules (Fig. 2). CTL 361A/21 was stimulated with autologous tumor cells in the presence of the indicated antibodies. MZ2-MEL is an HLA-B7-negative allogeneic melanoma cell line used as negative control. The production of TNF was measured after 18 h using the TNF-sensitive WEHI-164c13 cells (Gaugler et al., 1996). As shown in Fig. 2, the antigen recognized by CTL 361A/21 was presented by HLA-B7.

To isolate the nucleic acid molecule encoding the antigen recognized by CTL 361A/21, an oligo-dT-based cDNA library was used (see Gaugler et al., 1996). COS cells were co-transfected with DNA from pools of this library and with a plasmid containing the HLA-B7 cDNA. The transfected cells were screened with CTL 361A/21 by measuring TNF production using the assay as described above.

This experiment resulted in the isolation of cDNA 4.1 (SEQ ID NO:1), which was able to stimulate the CTL when transfected into COS cells together with the HLA-B7 cDNA (Fig. 2). This cDNA clone was 924 bp long. Its sequence was new and contained an open reading frame coding for a putative protein of 84 amino acids (SEQ ID NO:2).

To exclude the possibility that recognition of the transfected COS cells was an artefact due to high expression levels following transient transfection, an HLA-B7-positive sarcoma line (LB23-SAR) was transfected with an expression plasmid containing cDNA 4.1, and a stable transfectant was obtained. This transfectant, LB23-SAR+4.1, was recognized by CTL 361A/21 (Fig. 3). Chromium release was measured after 4 hours.

To identify the antigenic epitope recognized by CTL 361A/21, several synthetic peptides derived from SEQ ID NO:2 bearing a putative binding motif for HLA-B7 (Rammensee et al., *Immunogenetics* 41:178-228, 1995) were tested. One of these synthetic peptides, LPRWPPPQL (SEQ ID NO:3), was able to sensitize autologous EBV-B cells to

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lysis by CTL 361A/21 (Fig. 4). Chromium-labeled LE9211-EBV cells were incubated for 30 min at 37°C with the indicated concentrations of peptide. CTL 361A/21 was added at an effector to target ratio of 10, and chromium release was measured after 4 hours.

To determine the size of the full length messenger RNA, a Northern blot made with RNA from LE9211-RCC tumor cells was hybridized with a probe corresponding to cDNA 4.1. A messenger RNA of 2.2 kb was observed, suggesting that cDNA 4.1 was not complete. To find the missing 5' sequence, PCR amplification of the cDNA was performed using a primer corresponding to the 4.1 sequence and a 5' anchor primer, according to the 5'-RACE protocol (Gibco/BRL, Life Technologies, Gaithersburg, MD). This yielded a sequence fragment which allowed extension of the 4.1 sequence by 458 nucleotides at the 5' end, without modifying the open reading frame (Fig. 5).

Since the resulting sequence of 1382 bp was still substantially smaller than the messenger RNA, the cDNA library was screened by hybridization using cDNA 4.1 as a probe to isolate longer cDNA clones. One positive clone of 0.6 kb was obtained and 3 identical positive clones of 2.2 kb were obtained, of which clone I.1 is representative. The sequence of the small clone matched that of the 3' end of cDNA 4.1, but the sequence of clone I.1 surprisingly did not correspond to that of the positive strand of cDNA 4.1. The sequence of clone I.1 was new and contained an open reading frame coding for a putative protein of 476 amino acids (Fig. 6). This protein sequence does not contain antigenic peptide LPRWPPPQL. Accordingly, COS cells transfected with cDNA I.1 and with the HLA-B7 cDNA were not recognized by CTL 361A/21.

Because cDNA I.1 specifically hybridized with cDNA 4.1, the two sequences were compared in more detail, and it was found that the first 595 nucleotides of cDNA I.1 were identical to part of the antisense strand of 4.1. The remaining sequence was not related to cDNA 4.1. To further define the relationship between the two cDNAs, the corresponding genomic sequence was obtained. cDNA 4.1 was used as a probe to screen a phage library constructed with genomic DNA from LE9211-RCC cells. A positive phage was isolated which contained a 14kb insert, which was sequenced in the relevant part. The comparison of the three sequences revealed that cDNA I.1 corresponded to the fully spliced transcript of the gene, whereas cDNA 4.1 corresponded to an aberrant message that starts on the antisense strand of the first intron, is transcribed backwards on the antiparallel strand of exon 1 of cDNA I.1, and ends with a polyadenylation site that is located on the reverse strand of the

promoter (Fig. 7). This gene is therefore transcribed in both directions. The gene is referred to as RUR-1, which stands for renal tumor ubiquitous gene encoding a peptide on the reverse strand. The 2.2 kb transcript of RUR-1 corresponding to cDNA I.1 is presumably the normal message of this gene, whereas the RUR-1 message corresponding to cDNA 4.1 appears to

5 result from antisense transcription of the same gene.

The expression of these two transcripts in normal tissues was tested by RT-PCR. Expression of the RUR-1 sense message was assessed using primers VDE87 (5'-CCGTCAGGAACATCTACA-3'; SEQ ID NO:6) and VDE93 (5'-CCAACAGCCACATAAAAC-5'; SEQ ID NO:7) which are located in different exons

10 (Fig. 7). Expression of the RUR-1 antisense message was tested using primers VDE119 (5'-TAAATGGGTGGGCGGTGGGGGAGAC-3'; SEQ ID NO:8) and VDE120 (5'-TAGGCTGTTTGGAAAGGGTAGCACA-3'; SEQ ID NO:9) (Fig. 7). RT-PCR amplifications were performed in two steps. The reverse transcription was performed as described starting from 2 µg of total RNA (Van den Eynde et al., *J. Exp. Med.* 182:689-698,

15 1995). Aliquots corresponding to 50 ng of initial RNA were then used in a PCR reaction which was done as follows:

-H ₂ O	16.875 µl
-Buffer 10x (Takara)	2 µl
-dNTP (2.5 mM each)	2 µl
20 -primer 1 (20 µM)	0.5 µl
-primer 2 (20 µM)	0.5 µl
-Taq DNA polymerase (Takara, 5u/µl)	0.125 µl
-cDNA (corresponding to 50 ng of RNA)	2.5 µl

25 The PCR conditions were: 5 min at 94°C followed by 35 cycles consisting of 1 min at 94°C, 1 min at 54°C for primers VDE87/VDE93 or at 62°C for primers VDE119/VDE120, and 1 min at 72°C. A final elongation step was performed for 15 min at 72°C. PCR products were then visualized on agarose gels stained with ethidium bromide.

Because of their localization, primers VDE119 and VDE120 also amplified genomic

30 DNA that may be present in the RNA sample. Therefore, samples positive for RUR-1 antisense were retested after DNase treatment of the RNA, and only those that remained positive were considered to express the RUR-1 antisense message. Actin primers were always

used to check RNA integrity. It was found that the sense transcript of RUR-1 was expressed in all tissues tested, whereas the antisense transcript of RUR-1 was only present in kidney, liver and testis (Table 1).

In tumors, the antisense transcript was detected in all renal cell carcinoma samples, in a high proportion of colorectal carcinoma, melanoma, sarcoma and leukemia samples, and in small fractions of other tumors (Table 1).

Table 1. Expression of the sense and antisense transcripts of gene I.1¹

NORMAL TISSUES			TUMORS		
HISTOLOGICAL TYPE	Expression of Transcript		HISTOLOGICAL TYPE	Number of tumors expressing transcript	
	RUR-1 (sense)	RUR-1 (antisense)		RUR-1 (sense)	RUR-1 (antisense)
Adrenals	+	-	<u>Tumor Samples</u>		
Breast	+	-	Renal carcinomas	10/10	10/10
Colon	+	-	Colorectal carcinomas	15/15	14/15
Heart	±	-	Melanomas	21/24	15/24
Kidney	+	+	Sarcomas	5/9	7/9
Liver	+	+	Leukemias	5/18	12/18
Lung	+	-	Brain tumors	9/9	6/9
Ovary	+	-	Thyroid	4/5	4/5
Prostate	+	-	Mammary carcinomas	9/10	5/10
Skin	+	-	Prostatic carcinomas	7/10	4/10
Stomach	+	-	Oesophageal tumors	9/9	3/9
Testis	+	+	Bladder tumors	9/10	3/10
Urinary Bladder	+	± ²	Lung carcinomas	20/20	4/20
Uterus	+	-	Head & neck tumors	7/10	1/10
			Mesotheliomas	3/4	0/4

¹ Expression of RUR-1 sense transcript was tested by RT-PCR with primers VDE87 and VDE93; expression of RUR-1 antisense transcript was tested by RT-PCR with primers VDE119 and VDE120 (see Fig. 7).

² Bladder RNA was not treated with DNase, so the weak band detected could be due to contaminating DNA.

It also was confirmed that cell lines expressing the antisense transcript and HLA-B7 were recognized by CTL 361A/21 (Fig 8). Fig. 8A shows the lysis by CTL 361A/21 of two HLA-B7-positive renal cell carcinoma (RCC) lines expressing the RUR-1 antisense transcript. MZ1257-RCC and MZ1851-RCC are two RCC lines derived from different HLA-B7 patients. Chromium release was measured after 4 hours. Fig. 8B shows that TNF release by CTL 361A/21 was stimulated specifically by HLA-B7-positive tumor cells which express RUR-1 antisense cDNA. CTL 361A/21 was contacted with the indicated tumor cells and TNF production was measured 18h later. LY4-MEL, LB265-MEL, LB168-MEL and LB30-MEL are melanoma cell lines derived from four different HLA-B7-positive patients. The expression of the RUR-1 antisense transcript in the same cell lines was measured by RT-PCR using primers VDE119 and VDE 120.

Example 2: Identification of the alternative tumor rejection antigens encoded by RUR-1 antisense transcript.

On the basis of the findings made with the MAGE genes (and other genes) wherein multiple antigenic peptides are formed from the protein products of the gene, it is believed that the RUR-1 antisense cDNA can encode additional antigenic peptides presented by various HLA molecules.

At least two experimental approaches can be taken to identify additional tumor rejection antigens (e.g., other than SEQ ID NO:3) encoded by RUR-1 antisense cDNA. In a first method, CTL clones are generated by stimulating the peripheral blood lymphocytes (PBLs) of a patient with autologous normal cells transfected with RUR-1 antisense cDNA or with irradiated PBLs loaded with synthetic peptides corresponding to the putative proteins and matching the consensus for the appropriate HLA class I molecule to localize antigenic peptides within the RUR-1 antisense-encoded polypeptide (see, e.g., van der Bruggen et al., *Eur. J. Immunol.* 24:3038-3043, 1994; MAGE3 peptides presented by HLA.A2; Herman et al., *Immunogenetics* 43:377-383, 1996). This is the method described above. Localization of one or more antigenic peptides in a protein sequence can be aided by HLA peptide binding predictions made according to established rules for binding potential (e.g., Parker et al, *J. Immunol.* 152:163, 1994; Rammensee et al., *Immunogenetics* 41:178-228, 1995). HLA binding predictions can conveniently be made using an algorithm available via the Internet on

the National Institutes of Health World Wide Web site at URL <http://bimas.dcrt.nih.gov>.

Alternatively, CTL clones obtained by stimulation of lymphocytes with autologous tumor cells which express RUR-1 antisense cDNA are screened for specificity against COS cells transfected with RUR-1 antisense cDNA and autologous HLA alleles as described by Brichard et al. (*Eur. J. Immunol.* 26:224-230, 1996).

CTL recognition of RUR-1 antisense cDNA encoded peptides is determined by measuring release of TNF from the cytolytic T lymphocyte or by ^{51}Cr release assay (Herin et al., *Int. J. Cancer* 39:390-396, 1987) as described above. If a CTL clone specifically recognizes a transfected COS cell, shorter fragments of the coding sequences are tested to identify the region of the gene that encodes the peptide. Fragments of a RUR-1 antisense cDNA-encoded polypeptide are prepared by exonuclease III digestion or other standard molecular biology methods. Synthetic peptides are prepared to confirm the exact sequence of the antigen.

Optionally, shorter fragments of RUR-1 antisense cDNA are generated by PCR. Shorter fragments are used to provoke TNF release or ^{51}Cr release as above.

Synthetic peptides corresponding to portions of the shortest fragment of a RUR-1 antisense cDNA clone which provokes TNF release are prepared. Progressively shorter peptides are synthesized to determine the optimal RUR-1 antisense cDNA-encoded tumor rejection antigen peptides for a given HLA molecule.

Other aspects of the invention will be clear to the skilled artisan and need not be repeated here. All patents, published patent applications and literature references cited herein are incorporated by reference in their entirety.

While the invention has been described with respect to certain embodiments, it should be appreciated that many modifications and changes may be made by those of ordinary skill in the art without departing from the spirit of the invention. It is intended that such modification, changes and equivalents fall within the scope of the following claims.

What is claimed is:

CLAIMS

1. An isolated nucleic acid molecule selected from the group consisting of
 - (a) a nucleic acid molecule which codes for a RUR-1 sense-encoded or a RUR-1 antisense-encoded polypeptide and which hybridizes under stringent conditions to a molecule having a nucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO:1 and the nucleotide sequence of SEQ ID NO:4,
 - (b) deletions, additions and substitutions of the nucleic acid molecules of (a), which code for a a RUR-1 sense-encoded or a RUR-1 antisense-encoded polypeptide,
 - (c) nucleic acid molecules that differ from the nucleic acid molecules of (a) or (b) in codon sequence due to the degeneracy of the genetic code, and
 - (d) complements of (a), (b) and (c).
2. The isolated nucleic acid molecule of claim 1, wherein the isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:1.
3. The isolated nucleic acid molecule of claim 1, wherein the isolated nucleic acid molecule comprises the coding region of the nucleotide sequence of SEQ ID NO:1.
4. The isolated nucleic acid molecule of claim 1, wherein the isolated nucleic acid molecule consists of the nucleotide sequence of SEQ ID NO:1.
5. The isolated nucleic acid molecule of claim 1, wherein the isolated nucleic acid molecule comprises the nucleic acid sequence of SEQ ID NO:4.
6. The isolated nucleic acid molecule of claim 1 wherein the isolated nucleic acid molecule comprises the coding region of the nucleic acid sequence of SEQ ID NO:4.
7. The isolated nucleic acid molecule of claim 1, wherein the isolated nucleic acid molecule consists of the nucleotide sequence of SEQ ID NO:4.
8. An isolated nucleic acid molecule selected from the group consisting of:
 - (a) a unique fragment of nucleotides 1-1382 of SEQ ID NO:1 between 12 and 1381

contiguous nucleotides in length,

(b) a unique fragment of nucleotides 1-2167 of SEQ ID NO:4 between 12 and 2166 contiguous nucleotides in length,

(c) complements of "(a)", and

(d) complements of "(b)",

wherein the unique fragment excludes nucleic acid molecules which consist only of a nucleotide sequence selected from the group consisting of SEQ ID NO:10 and SEQ ID NO:11.

9. The isolated nucleic acid molecule of claim 8, wherein the isolated nucleic acid molecule is selected from the group consisting of nucleic acid molecules having at least 14 contiguous nucleotides, 15 contiguous nucleotides, 16 contiguous nucleotides, 18 contiguous nucleotides, 20 contiguous nucleotides, 22 contiguous nucleotides and 25 contiguous nucleotides.

10. The isolated nucleic acid molecule of claim 8, wherein the isolated nucleic acid molecule is between 12 and 32 contiguous nucleotides.

11. The isolated nucleic acid molecule of claim 8, wherein the isolated nucleic acid molecule comprises at least 5 contiguous nucleotides not present in SEQ ID NO:10 or SEQ ID NO:11.

12. An expression vector comprising the isolated nucleic acid molecule of any of claims 1-11 operably linked to a promoter.

13. A host cell transformed or transfected with the expression vector of claim 12.

14. The host cell of claim 13, wherein the host cell expresses an HLA molecule.

15. An isolated polypeptide encoded by the isolated nucleic acid molecule of any of claims 1-7, or a functional variant thereof having additions, deletions or substitutions in the amino acid sequence of the isolated polypeptide.

16. The isolated polypeptide of claim 15, wherein the polypeptide has an amino acid sequence selected from the group consisting of the amino acid sequence of SEQ ID NO:2, the amino acid sequence of SEQ ID NO:3 and the amino acid sequence of SEQ ID NO:5.

17. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:3.

18. An isolated polypeptide selected from the group consisting of:

(a) a unique fragment of SEQ ID NO:2 between 9 and 83 amino acids in length, and

(b) a unique fragment of SEQ ID NO:5 between 9 and 475 amino acids in length.

19. The isolated polypeptide of claim 18, wherein the unique fragment binds to a polypeptide-binding agent.

20. The isolated polypeptide of claim 19, wherein the polypeptide-binding agent is an antibody or a cytotoxic T lymphocyte.

21. An isolated polypeptide which selectively binds a protein encoded by the isolated nucleic acid molecule of any of claims 1-7.

22. The isolated polypeptide of claim 21, wherein the isolated polypeptide is an Fab or F(ab)₂ fragment of an antibody.

23. The isolated polypeptide of claim 21, wherein the isolated polypeptide is a fragment of an antibody, the fragment including a CDR3 region selective for the protein.

24. The isolated polypeptide of claim 21, wherein the isolated polypeptide is a monoclonal antibody.

25. A kit for detecting the presence of the expression of a nucleic acid which encodes a tumor associated polypeptide precursor, comprising a pair of isolated nucleic acid molecules each of which consists of a molecule selected from the group consisting of (a) a 12-32 nucleotide contiguous segment of nucleotides 1-1382 of SEQ ID NO:1, and (b) complements

of "(a)", wherein the contiguous segments are nonoverlapping.

26. The kit of claim 25, a wherein the pair of isolated nucleic acid molecules is constructed and arranged to selectively amplify an isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1.

27. The kit of claim 26, wherein the pair of isolated nucleic acid molecules is SEQ ID NO:8 and SEQ ID NO:9.

28. The kit of claim 25 wherein the pair of isolated nucleic acid molecules is PCR primers, wherein one of the primers is a unique fragment of SEQ ID NO:1.

29. A method for diagnosing a disorder characterized by expression of a RUR-1 antisense cDNA nucleic acid molecule or an expression product thereof, comprising:

contacting a biological sample isolated from a subject with an agent that selectively binds the isolated RUR-1 antisense cDNA nucleic acid molecule of claim 1 or an expression product thereof, and

determining the interaction between the agent and the nucleic acid molecule or the expression product as a determination of the disorder.

30. The method of claim 29 wherein the agent is a nucleic acid molecule comprising SEQ ID NO:1 or a unique fragment thereof.

31. The method of claim 29 wherein the interaction is determined by amplifying at least a portion of the nucleic acid molecule.

32. The method of claim 29 wherein the agent is a cytolytic T lymphocyte.

33. The method of claim 29 wherein the agent is an antibody or antibody fragment.

34. The method of claim 29 wherein the biological sample is isolated from a tissue selected from the group consisting of non-liver tissue, non-kidney tissue, non-bladder tissue,

and non-testis tissue.

35. A method for treating a subject with a disorder characterized by expression of a
RUR-1 antisense cDNA-encoded tumor associated polypeptide, comprising
5 administering to the subject an amount of an agent which enriches selectively in the
subject the presence of complexes of a HLA molecule and a tumor rejection antigen derived
from a RUR-1 antisense cDNA-encoded tumor associated polypeptide, sufficient to
ameliorate the disorder.

10 36. The method of claim 35, wherein the agent is an isolated polypeptide comprising the
amino acid sequence of SEQ ID NO:3, or an immunogenic fragment thereof.

37. The method of claim 35, wherein the disorder is cancer excluding cancers selected
from the group consisting of liver cancer, kidney cancer, bladder cancer, and testicular cancer.

15 38. A method for treating a subject with a disorder characterized by expression of a
RUR-1 antisense cDNA nucleic acid molecule or an expression product thereof, comprising:
administering to the subject an amount of autologous cytotoxic T cells sufficient to
ameliorate the disorder, wherein the cytotoxic T cells are specific for complexes of an HLA
20 molecule and a RUR-1 antisense cDNA-encoded tumor associated polypeptide or an
immunogenic fragment thereof.

39. The method of claim 38, wherein the RUR-1 antisense cDNA-encoded tumor
associated polypeptide comprises the amino acid sequence of SEQ ID NO:3.

25 40. The method of claim 39, wherein the disorder is cancer excluding cancers selected
from the group consisting of liver cancer, kidney cancer, bladder cancer, and testicular cancer.

41. A method for treating a subject with a disorder characterized by expression of a
30 RUR-1 antisense cDNA nucleic acid molecule or an expression product thereof, comprising:
administering to the subject an amount of a RUR-1 antisense cDNA-encoded tumor
associated polypeptide or an immunogenic fragment thereof sufficient to ameliorate the

disorder.

42. The method of claim 41, wherein the RUR-1 antisense cDNA-encoded tumor associated polypeptide comprises the amino acid sequence of SEQ ID NO:3.

43. The method of claim 42, wherein the disorder is cancer excluding cancers selected from the group consisting of liver cancer, kidney cancer, bladder cancer, and testicular cancer.

44. A method for enriching selectively a population of T cells with cytotoxic T cells specific for a RUR-1 antisense cDNA-encoded tumor associated polypeptide comprising:
contacting an isolated population of T cells with an agent presenting a complex of a RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof and a HLA presenting molecule in an amount sufficient to selectively enrich the isolated population of T cells with the cytotoxic T cells.

45. The method of claim 44 wherein the RUR-1 antisense cDNA-encoded tumor associated polypeptide comprises the amino acid sequence of SEQ ID NO:3.

46. The method of claim 45 wherein the agent is a cell which expresses a RUR-1 antisense cDNA-encoded tumor associated polypeptide and a HLA molecule.

47. A vaccine composition comprising a nucleic acid encoding a RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof.

48. The vaccine composition of claim 47, further comprising a nucleic acid encoding a second tumor associated polypeptide or an immunogenic fragment thereof which is a non-RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof.

49. A vaccine composition comprising a RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof.

50. The vaccine composition of claim 49, wherein the RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof comprises the amino acid sequence of SEQ ID NO:3.

5 51. The vaccine composition of claim 49, further comprising a second tumor associated polypeptide or an immunogenic fragment thereof which is a non-RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof.

10 52. A vaccine composition comprising a cell which expresses a RUR-1 antisense cDNA nucleic acid or polypeptide, or an immunogenic fragment thereof.

15 53. The vaccine composition of claim 52, the cell further comprising a second tumor associated nucleic acid or polypeptide, or an immunogenic fragment thereof, which is a non-RUR-1 antisense cDNA nucleic acid or tumor associated polypeptide, or an immunogenic fragment thereof.

54. The vaccine composition of any of claims 47-53, further comprising an adjuvant.

20 55. The vaccine composition of any of claims 47-53, further comprising a pharmaceutically acceptable carrier.

25 56. An isolated nucleic acid molecule comprising a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:3, or an immunogenic fragment thereof.

30 57. The isolated nucleic acid molecule of claim 56, further comprising a nucleotide sequence encoding a second tumor associated polypeptide or an immunogenic fragment thereof which is a non-RUR-1 antisense cDNA-encoded tumor associated polypeptide or an immunogenic fragment thereof.

58. A composition comprising:
the isolated nucleic acid as claimed in claim 1, and

a pharmaceutically acceptable carrier.

59. A composition comprising:
the isolated polypeptide as claimed in claim 15, and
a pharmaceutically acceptable carrier.

60. A method for determining the prognosis of a disorder characterized by expression of a RUR-1 antisense nucleic acid molecule or an expression product thereof, comprising:

(a) contacting a biological sample isolated from a subject at a first time with an agent that selectively binds the isolated RUR-1 antisense nucleic acid molecule of claim 1 or an expression product thereof,

(b) determining the interaction between the agent and the nucleic acid molecule or the expression product as a determination of the state of the disorder at the first time,

(c) contacting a second biological sample isolated from the subject at a second time with the agent,

(d) determining the interaction between the agent and the nucleic acid molecule or the expression product in the second biological sample as a determination of the state of the disorder at the second time, and

(e) comparing the state of the disorder at the first time and the second time as a determination of the prognosis.

61. The method of claim 60 wherein the agent is a nucleic acid molecule comprising SEQ ID NO:1 or a unique fragment thereof.

62. The method of claim 60 wherein the interactions are determined by amplifying at least a portion of the nucleic acid molecule.

63. The method of claim 60 wherein the agent is a cytolytic T lymphocyte.

64. The method of claim 60 wherein the agent is an antibody or antibody fragment.

65. The method of claim 60 wherein the biological samples are isolated from a tissue

selected from the group consisting of non-liver tissue, non-kidney tissue, non-bladder tissue, and non-testis tissue.

Pub. No. 99/04360

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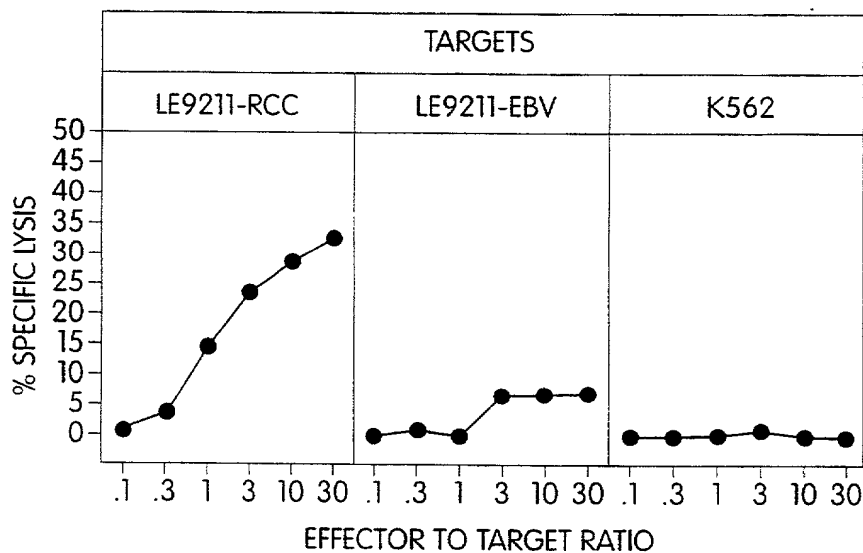


Fig. 1

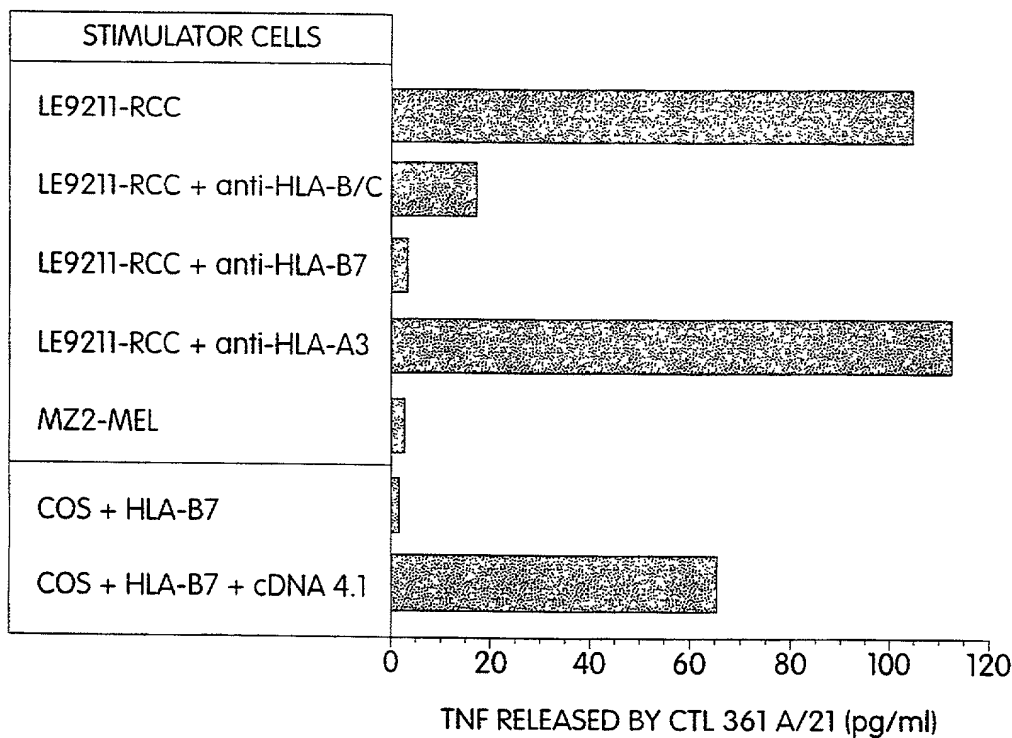


Fig. 2

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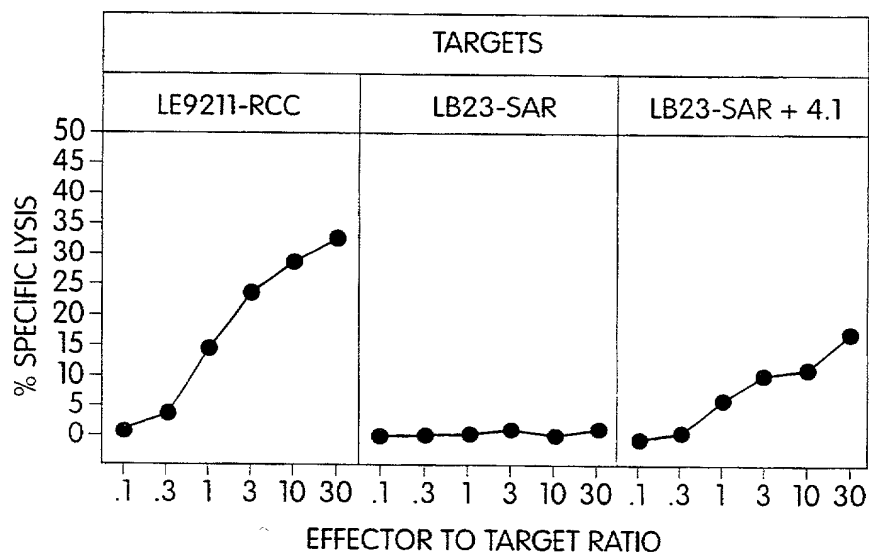


Fig. 3

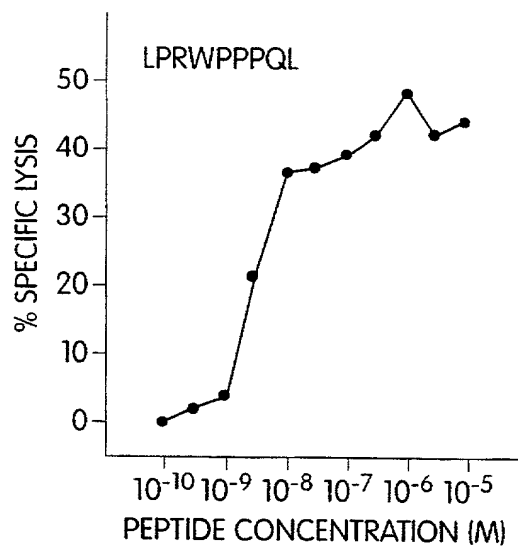


Fig. 4

Fig. 5

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 212 ATCCCCGGCCCCTGGGTGCGGTGAGGGCCGCTGGACAGCGGAGGTGCTCCGACGCGAAGCAGCGAGCTGAGGGCGCGGCAGCGGGGAAG ATG AGC GGC
 312 AGC AGC GCC AGG TCC AGC CAC CTG TCT CAG CCC GTC GTG AAG AGC GTG CTT GTG TAC CGC AAC GGG GAC CCC TTC TAC
 390 GCG GGG CGC GTC GTC ATC CAT GAG AAG GTG TCC AGC TTC GAA GTC TTC CTG AAG GAG GTG ACC GGC GGC GTT
 468 CAG GCA CCC TTT GGG GCC GTC AGG AAC ATC TAC ACC CCG CGG ACT GGC CAC CGA ATC CGG AAG ATA GAC CAG ATC CAG
 546 AGC GGG GGC AAT TAC GTG GGT GGA GGC GAA GCC TTC AAG AAA CTC AAT TAC TTG GAC ATA GGA GAA ATC AAG AAA
 624 AGA CCA ATG GAA GTT GTT AAT ACA GAG GTA AAA CCA GTA ATC CAT AGC AGG ATC AAC GTG TCA GCT CGC TTT AGA AAA
 702 CCG CTT CAG GAG CCG TGC ACT ATC TTC TTG ATT GCA AAT GGA GAC CTC ATA AAC CCA GCT TCT CGC CTC CTT ATC CCC
 780 AGA AAA ACC TTG AAT CAG TGG GAT CAT GTA CTA CAA ATG GTC ACA GAA AAA ATC ACT CTG AGG AGC GGG GCT GTT CAC
 858 AGG CTT TAT ACT TTA GAA GGA AAA CTT GTT GAG AGT GGA GCA GAG TTG GAG AAT GGG CAG TTT TAT GTG GCT GTT GGC
 936 AGA GAT AAG TTT AAG AAA CTG CCT TAC GGT GAG TTA CTT TTT GAC AAG TCA ACG ATG AGA AGG CCT TTT GGT CAG AAA
 1014 GCT TCT TCA CTA CCT CCT ATT GTA GGA TCC AGA AAG TCT AAA GGG AGT GGA AAT GAT CGC CAC TCT AAG TCA ACA GTT
 1092 GGA TCC AGT GAC AAC TCA TCT CCT CAG CCC CTG AAG AGG AAA GGG AAA GAA GAC GTG AAT TCA GAA AAA CTG ACG

Fig. 6

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1170 AAA TTG AAA CAA AAT GTA AAA TTA AAG AAT TCA CAA ACC ATT CCA AAT AGT GAT GAA GGC ATT TTC AAA GCT GGA
 K L K Q N V K L K N S Q E T I P N S D E G I F K A G
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 A E R S E T R G A A E V Q E D E D T Q V E V P V D Q
 1326 AGG CCA GCA GAA ATA GTA GAC GAG GAA GAA GAT GGA AAG GCA AAC AAG GAT GCA GAA CAG AAA GAA GAC TTT TCA
 R P A E I V D E E D G E K A N K D A E Q K E D F S
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 H S E Q Q A R P A R V N G G T D E E N G E E L Q Q V
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Fig. 6 cont.

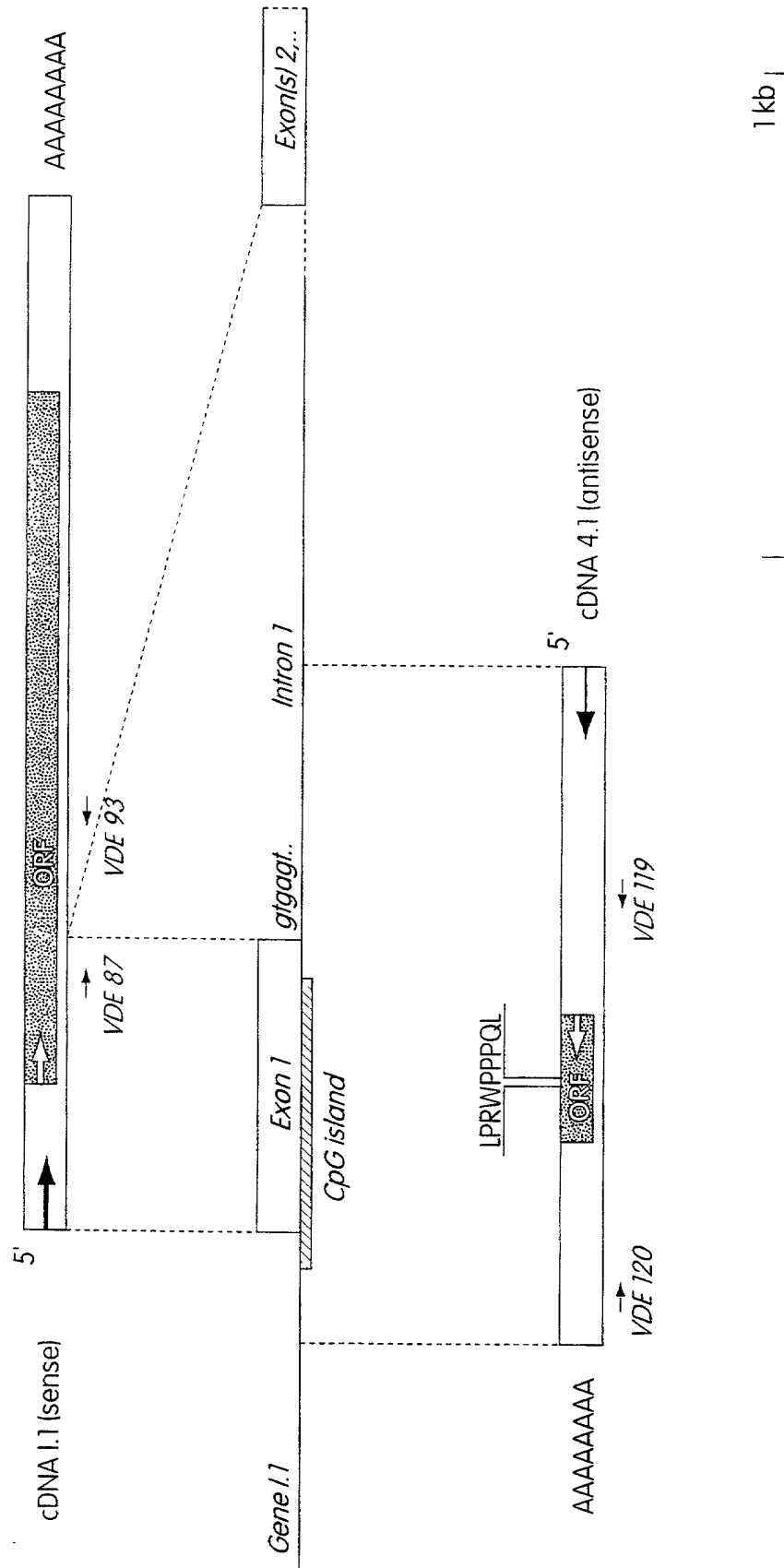


Fig. 7

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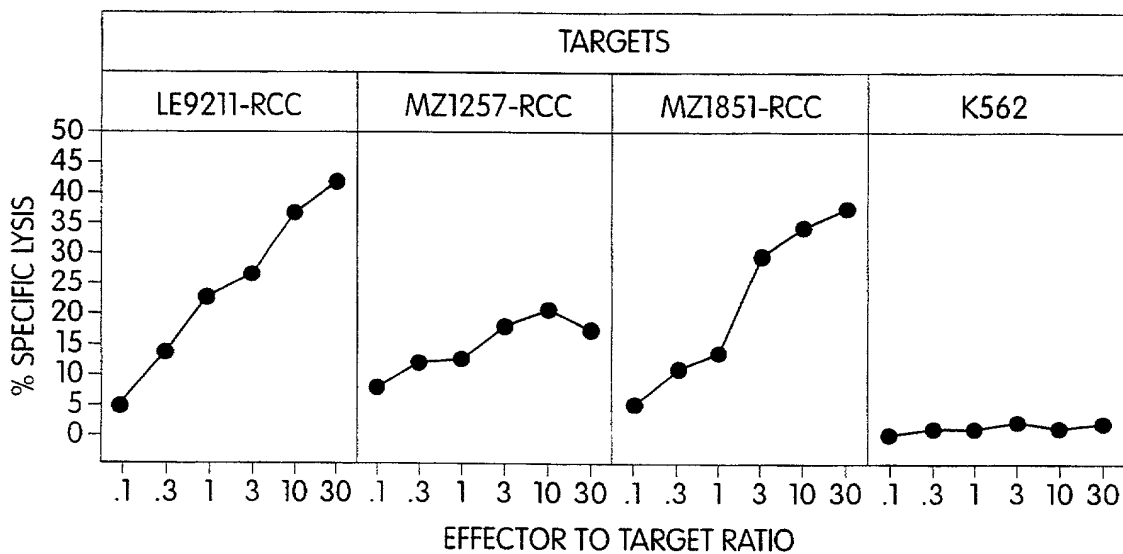


Fig. 8A

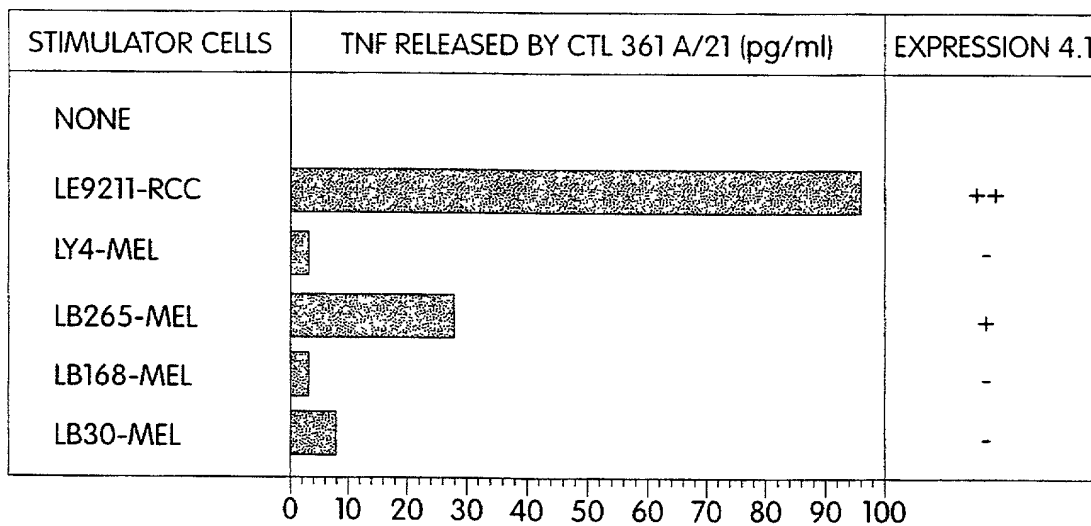


Fig. 8B

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled

**TUMOR ASSOCIATED ANTIGEN ENCODED BY THE REVERSE STRAND OF A NEW
UBIQUITOUSLY EXPRESSED GENE**

the specification of which is attached hereto unless the following is checked:

[X] was filed on May 13, 1999, as Application No. PCT/US99/10424, bearing attorney docket no. L0461/7034WO, and was amended on November 2, 2000.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or section 365(a) of any PCT International application designating at least one country other than the United States listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed:

Prior Foreign PCT International Application(s) and any priority claims under 35 U.S.C. §§119 and 365(a),(b):

			Priority Claimed	
			<input type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country-if PCT, so indicate)	(DD/MM/YY Filed)	YES	NO
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country-if PCT, so indicate)	(DD/MM/YY Filed)	YES	NO
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>
(Number)	(Country-if PCT, so indicate)	(DD/MM/YY Filed)	YES	NO

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

<u>60/085,318</u>	<u>13 May 1998</u>
(Application Number)	(filing date)
_____	_____
(Application Number)	(filing date)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application No.)	(filing date)	(status-patented, pending, abandoned)
(Application No.)	(filing date)	(status-patented, pending, abandoned)

PCT International Applications designating the United States:

PCT/US99/10424		13 May 1999	Pending
(PCT Appl. No.)	(U.S. Ser. No.)	(PCT filing date)	(status-patented,pending,abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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Eric Amundsen	46,518	Robert E. Hunt	39,231	Christopher S. Schultz	37,929
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c/o Wolf, Greenfield & Sacks, P.C.,
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600 Atlantic Avenue
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment,

or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

100
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200
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November 23, 2000

Date

- 1 -

SEQUENCE LISTING

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<120> TUMOR ASSOCIATED ANTIGEN ENCODED BY THE REVERSE STRAND
OF A NEW UBIQUITOUSLY EXPRESSED GENE

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ggatctgcat	ttcttcatat	caacccccaca	ctatagggca	cctaaatggg	tgggcggtgg	540
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ctggacacct	tcttctc	atg	gat	gac	gac	770
		Met	Asp	Asp	Asp	
		1		5		
					10	
gtc	ccc	gtt	gcg	gta	cac	818
Val	Pro	Val	Ala	Val	His	
		15		20		
gtg	gct	gga	cct	ggc	gct	866
Val	Ala	Gly	Pro	Gly	Ala	
		30		35		
cct	cag	ctc	gct	gct	tcg	914
Pro	Gln	Leu	Ala	Ala	Ser	
		45		50		
cct	cac	cg	acc	cag	ggc	962
Pro	His	Arg	Thr	Gln	Gly	
		60		65		
ctg	acg	aat	cca	cag	gtg	1016
Leu	Thr	Asn	Pro	Gln	Val	
				Lys	Glu	
					80	

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cagaggagac actaggagct tgcaggactc ggagtagacg ctcaagtttt tcaccgtggc 1076
gtgcacagcc aatcaggacc cgcagtgcgc gcaccacacc aggttcacct gctacgggca 1136
gaatcaaggt ggacagcttc tgagcaggag ccggaacgc gcggggcctt caaacaggca 1196
cgcctagtga gggcaggaga gaggaggacg cacacacaca cacacacaca aatatgggta 1256
aaccgaattt cttacatcat atctgtgcta ccctttccaa acagcctaata ttttcttttc 1316
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<212> PRT

<213> Homo sapiens

<400> 2

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His Lys His Ala Leu His Asp Gly Leu Arg Gln Val Ala Gly Pro Gly
20          25          30
Ala Ala Ala Ala His Leu Pro Arg Trp Pro Pro Pro Gln Leu Ala Ala
35          40          45
Ser Arg Arg Glu Ala Pro Pro Leu Ser Gln Arg Pro His Arg Thr Gln
50          55          60
Gly Ala Gly Ser Pro Pro Glu Thr Asn Glu Lys Leu Thr Asn Pro Gln
65          70          75          80
Val Lys Glu Lys

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<210> 3

<211> 9

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<213> Homo sapiens

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Leu Pro Arg Trp Pro Pro Pro Gln Leu
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tagtgtctcc tctgggtgga cgcctaggcg caggccggtt acttctcttt cacctgtgga 180
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tggaacagcg gaggtgcctc ccgacgcgaa gcagcgagct gaggcggcgg ccagcgggga 300
ag atg agc ggc agc agc gcc agg tcc agc cac ctg tct cag ccc gtc 347
Met Ser Gly Ser Ser Ala Arg Ser Ser His Leu Ser Gln Pro Val
1          5          10          15
gtg aag agc gtg ctt gtg tac cgc aac ggg gac ccc ttc tac gcg ggg 395
Val Lys Ser Val Leu Val Tyr Arg Asn Gly Asp Pro Phe Tyr Ala Gly
20          25          30
cgc cgc gtc gtc atc cat gag aag aag gtg tcc agc ttc gaa gtc ttc 443

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Arg	Arg	Val	Val	Ile	His	Glu	Lys	Lys	Val	Ser	Ser	Phe	Glu	Val	Phe		
		35						40					45				
ctg	aag	gag	gtg	acc	ggc	ggc	gtt	cag	gca	ccc	ttt	ggg	gcc	gtc	agg		491
Leu	Lys	Glu	Val	Thr	Gly	Gly	Val	Gln	Ala	Pro	Phe	Gly	Ala	Val	Arg		
		50					55					60					
aac	atc	tac	acc	ccg	cgg	act	ggc	cac	cga	atc	cgg	aag	cta	gac	cag		539
Asn	Ile	Tyr	Thr	Pro	Arg	Thr	Gly	His	Arg	Ile	Arg	Lys	Leu	Asp	Gln		
	65					70				75							
atc	cag	agc	ggg	ggc	aat	tac	gtg	gct	gga	ggc	cag	gaa	gcc	ttc	aag		587
Ile	Gln	Ser	Gly	Gly	Asn	Tyr	Val	Ala	Gly	Gly	Gln	Glu	Ala	Phe	Lys		
80					85					90				95			
aaa	ctc	aat	tac	ttg	gac	ata	gga	gaa	atc	aag	aaa	aga	cca	atg	gaa		635
Lys	Leu	Asn	Tyr	Leu	Asp	Ile	Gly	Glu	Ile	Lys	Lys	Arg	Pro	Met	Glu		
			100					105				110					
gtt	gtt	aat	aca	gag	gta	aaa	cca	gta	atc	cat	agc	agg	atc	aac	gtg		683
Val	Val	Asn	Thr	Glu	Val	Lys	Pro	Val	Ile	His	Ser	Arg	Ile	Asn	Val		
		115						120				125					
tca	gct	cgc	ttt	aga	aaa	ccg	ctt	cag	gag	ccg	tgc	act	atc	ttc	ttg		731
Ser	Ala	Arg	Phe	Arg	Lys	Pro	Leu	Gln	Glu	Pro	Cys	Thr	Ile	Phe	Leu		
		130				135					140						
att	gca	aat	gga	gac	ctc	ata	aac	cca	gct	tct	cgc	ctc	ctt	atc	ccc		779
Ile	Ala	Asn	Gly	Asp	Leu	Ile	Asn	Pro	Ala	Ser	Arg	Leu	Leu	Ile	Pro		
	145					150					155						
aga	aaa	acc	ttg	aat	cag	tgg	gat	cat	gta	cta	caa	atg	gtc	aca	gaa		827
Arg	Lys	Thr	Leu	Asn	Gln	Trp	Asp	His	Val	Leu	Gln	Met	Val	Thr	Glu		
160					165					170				175			
aaa	atc	act	ctg	agg	agc	ggg	gct	gtt	cac	agg	ctt	tat	act	tta	gaa		875
Lys	Ile	Thr	Leu	Arg	Ser	Gly	Ala	Val	His	Arg	Leu	Tyr	Thr	Leu	Glu		
			180			185					190						
gga	aaa	ctt	gtt	gag	agt	gga	gca	gag	ttg	gag	aat	ggg	cag	ttt	tat		923
Gly	Lys	Leu	Val	Glu	Ser	Gly	Ala	Glu	Leu	Glu	Asn	Gly	Gln	Phe	Tyr		
		195				200					205						
gtg	gct	gtt	ggc	aga	gat	aag	ttt	aag	aaa	ctg	cct	tac	ggt	gag	tta		971
Val	Ala	Val	Gly	Arg	Asp	Lys	Phe	Lys	Lys	Leu	Pro	Tyr	Gly	Glu	Leu		
		210				215					220						
ctt	ttt	gac	aag	tca	acg	atg	aga	agg	cct	ttt	ggt	cag	aaa	gct	tct		1019
Leu	Phe	Asp	Lys	Ser	Thr	Met	Arg	Arg	Pro	Phe	Gly	Gln	Lys	Ala	Ser		
		225				230				235							
tca	cta	cct	cct	att	gta	gga	tcc	aga	aag	tct	aaa	ggg	agt	gga	aat		1067
Ser	Leu	Pro	Pro	Ile	Val	Gly	Ser	Arg	Lys	Ser	Lys	Gly	Ser	Gly	Asn		
240					245					250				255			
gat	cgc	cac	tct	aag	tca	aca	gtt	gga	tcc	agt	gac	aac	tca	tct	cct		1115
Asp	Arg	His	Ser	Lys	Ser	Thr	Val	Gly	Ser	Ser	Asp	Asn	Ser	Ser	Pro		
			260					265				270					
cag	ccc	ctg	aag	agg	aaa	ggg	aaa	aaa	gaa	gac	gtg	aat	tca	gaa	aaa		1163
Gln	Pro	Leu	Lys	Arg	Lys	Gly	Lys	Lys	Glu	Asp	Val	Asn	Ser	Glu	Lys		
			275					280				285					
ctg	acg	aaa	ttg	aaa	caa	aat	gta	aaa	tta	aag	aat	tca	caa	gaa	acc		1211
Leu	Thr	Lys	Leu	Lys	Gln	Asn	Val	Lys	Leu	Lys	Asn	Ser	Gln	Glu	Thr		
		290					295				300						
att	cca	aat	agt	gat	gaa	ggc	att	ttc	aaa	gct	gga	gca	gag	agg	tct		1259
Ile	Pro	Asn	Ser	Asp	Glu	Gly	Ile	Phe	Lys	Ala	Gly	Ala	Glu	Arg	Ser		
		305				310				315							
gaa	aca	cgg	ggg	gca	gca	gaa	gtc	caa	gaa	gat	gaa	gat	act	cag	gtt		1307
Glu	Thr	Arg	Gly	Ala	Ala	Glu	Val	Gln	Glu	Asp	Glu	Asp	Thr	Gln	Val		
320					325					330				335			
gag	gtt	cca	gtc	gat	cag	agg	cca	gca	gaa	ata	gta	gac	gag	gaa	gaa		1355

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Glu Val Pro Val Asp Gln Arg Pro Ala Glu Ile Val Asp Glu Glu Glu
 340 345 350
 gat gga gag aag gca aac aag gat gca gaa cag aaa gaa gac ttt tca 1403
 Asp Gly Glu Lys Ala Asn Lys Asp Ala Glu Gln Lys Glu Asp Phe Ser
 355 360 365
 gga atg aat ggt gac ctt gaa gag gaa gga ggt agg gag gct aca gat 1451
 Gly Met Asn Gly Asp Leu Glu Glu Glu Gly Gly Arg Glu Ala Thr Asp
 370 375 380
 gcc cct gag caa gtc gag gag att ctg gat cac agt gag cag cag gca 1499
 Ala Pro Glu Gln Val Glu Glu Ile Leu Asp His Ser Glu Gln Gln Ala
 385 390 395
 cgc cct gct cgt gta aat gga ggc acc gat gag gag aat ggt gag gag 1547
 Arg Pro Ala Arg Val Asn Gly Gly Thr Asp Glu Glu Asn Gly Glu Glu
 400 405 410 415
 ctg cag cag gtt aat aat gag ctt caa ctg gtc cta gac aag gaa aga 1595
 Leu Gln Gln Val Asn Asn Glu Leu Gln Leu Val Leu Asp Lys Glu Arg
 420 425 430
 aag tct caa gga gct ggc agt gga caa gat gag gct gat gta gac cct 1643
 Lys Ser Gln Gly Ala Gly Ser Gly Gln Asp Glu Ala Asp Val Asp Pro
 435 440 445
 caa aga cca cca agg cca gaa gta aaa att acc agt cca gaa gaa aat 1691
 Gln Arg Pro Pro Arg Pro Glu Val Lys Ile Thr Ser Pro Glu Glu Asn
 450 455 460
 gaa aac aac caa caa aac aag gac tat gct gcc gtg gct tagaagattt tt 1742
 Glu Asn Asn Gln Gln Asn Lys Asp Tyr Ala Ala Val Ala
 465 470 475
 aaaaagagag tatatggatc gcaagaaaaa tgaagggtta tcataactga aagataagca 1802
 catagttatt gctgaatata atgtgacact atggtcgaat actacctacg aattataaca 1862
 ttagaagcct agtggaaga ccagataact ttaaagggt actaaaggat aattacttac 1922
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 cgctttatta taagtttaga aggcagttta ttctaaataa tttttctcta ggaaggcgta 2102
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 35 40 45
 Lys Glu Val Thr Gly Gly Val Gln Ala Pro Phe Gly Ala Val Arg Asn
 50 55 60
 Ile Tyr Thr Pro Arg Thr Gly His Arg Ile Arg Lys Leu Asp Gln Ile
 65 70 75 80
 Gln Ser Gly Gly Asn Tyr Val Ala Gly Gly Gln Glu Ala Phe Lys Lys
 85 90 95
 Leu Asn Tyr Leu Asp Ile Gly Glu Ile Lys Lys Arg Pro Met Glu Val
 100 105 110
 Val Asn Thr Glu Val Lys Pro Val Ile His Ser Arg Ile Asn Val Ser
 115 120 125

- 5 -

Ala Arg Phe Arg Lys Pro Leu Gln Glu Pro Cys Thr Ile Phe Leu Ile
 130 135 140
 Ala Asn Gly Asp Leu Ile Asn Pro Ala Ser Arg Leu Leu Ile Pro Arg
 145 150 155 160
 Lys Thr Leu Asn Gln Trp Asp His Val Leu Gln Met Val Thr Glu Lys
 165 170 175
 Ile Thr Leu Arg Ser Gly Ala Val His Arg Leu Tyr Thr Leu Glu Gly
 180 185 190
 Lys Leu Val Glu Ser Gly Ala Glu Leu Glu Asn Gly Gln Phe Tyr Val
 195 200 205
 Ala Val Gly Arg Asp Lys Phe Lys Lys Leu Pro Tyr Gly Glu Leu Leu
 210 215 220
 Phe Asp Lys Ser Thr Met Arg Arg Pro Phe Gly Gln Lys Ala Ser Ser
 225 230 235 240
 Leu Pro Pro Ile Val Gly Ser Arg Lys Ser Lys Gly Ser Gly Asn Asp
 245 250 255
 Arg His Ser Lys Ser Thr Val Gly Ser Ser Asp Asn Ser Ser Pro Gln
 260 265 270
 Pro Leu Lys Arg Lys Gly Lys Lys Glu Asp Val Asn Ser Glu Lys Leu
 275 280 285
 Thr Lys Leu Lys Gln Asn Val Lys Leu Lys Asn Ser Gln Glu Thr Ile
 290 295 300
 Pro Asn Ser Asp Glu Gly Ile Phe Lys Ala Gly Ala Glu Arg Ser Glu
 305 310 315 320
 Thr Arg Gly Ala Ala Glu Val Gln Glu Asp Glu Asp Thr Gln Val Glu
 325 330 335
 Val Pro Val Asp Gln Arg Pro Ala Glu Ile Val Asp Glu Glu Glu Asp
 340 345 350
 Gly Glu Lys Ala Asn Lys Asp Ala Glu Gln Lys Glu Asp Phe Ser Gly
 355 360 365
 Met Asn Gly Asp Leu Glu Glu Glu Gly Gly Arg Glu Ala Thr Asp Ala
 370 375 380
 Pro Glu Gln Val Glu Glu Ile Leu Asp His Ser Glu Gln Gln Ala Arg
 385 390 395 400
 Pro Ala Arg Val Asn Gly Gly Thr Asp Glu Glu Asn Gly Glu Glu Leu
 405 410 415
 Gln Gln Val Asn Asn Glu Leu Gln Leu Val Leu Asp Lys Glu Arg Lys
 420 425 430
 Ser Gln Gly Ala Gly Ser Gly Gln Asp Glu Ala Asp Val Asp Pro Gln
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18

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<400> 7
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 aacctctacc ccccaactgc aacgagagtt ttgaggggca tcaatcacac cgagaagtca 180
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actcttgaat	gaacagattg	cgatttactt	tcagacataa	tttggagatg	gcagtagatc	240
anaatgtgtc	catgacttgt	taacatgcct	ttccgttctt	cctccttaag	ccaaaatcca	300
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tcaggagtgt	tagtgactag	tatcgccatt	gccgtccgct	taagtgcfff	gcaggctffg	420
catngcttng	ttggctccca	atgnngcgac	gctcaggaag	gaagttgttn	aagggnagnac	480
ccngttanag	tttataaagc	ctggatggta	tggttngccg	agtaatgnga	aatcctgtgg	540
gantttccac	tgatccaggt	ccaatcttta	cccagtagnt	atctctctcc	ntctccctta	600
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